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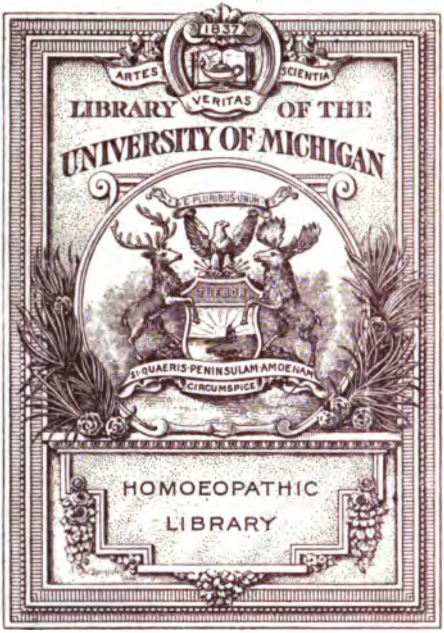
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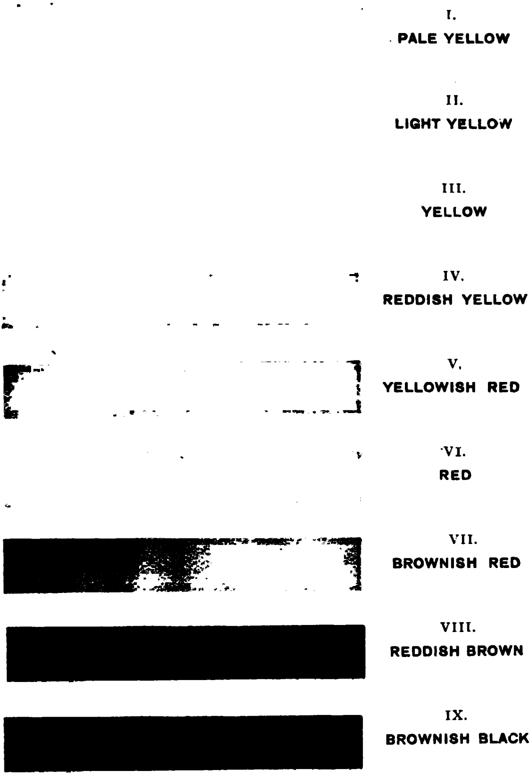

Scale of urinary colors, according to Vogel.

A MANUAL OF LABORATORY METHODS

BY

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ILLUSTRATED

BURTON PUBLISHING COMPANY
MEDICAL PUBLISHERS
KANSAS CITY, MISSOURI
1909.

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TO MY CLASSES

WHO HAVE ENCOURAGED ME BY THEIR RESPECT AND LOYALTY THIS VOLUME IS FRATERNALLY DEDICATED.

PREFACE.

This little book is written to meet the needs of an exacting class room and laboratory work. There is no attempt at explanation or elaboration. The sole aim is to state laboratory processes as consecutive steps. This is done in a few words.

The work covers the methods found useful in a medical, clinical and industrial laboratory experience of twelve years. The writer is a firm believer in the use of approximate quantitative methods which are sufficiently accurate for clinical purposes and has not hesitated to give them prominent place. The accurate quantitative methods are also given. Many modifications and methods not found in the text books are included.

It is hoped that this little manual will help the busy physician as the methods given have helped the class worker.

The writer is indebted to Dr. J. L. R. Marsh for kindly suggestions and for proof reading.

E. M. PERDUE.

Kansas City, Aug., 1909.

CONTENTS.

CHAPTER I.

HISTOLOGY AND PATHOLOGY.

The Preparation of Tissue — Hardening — Embedding—Blocking—Cutting and Staining—Double Staining and Mounting—Method with Hematoxylin and Eosin—Van Gieson's Method—Silver Nitrate Method—Cutting Frozen Sections—Staining Fat—Osmic Acid Method—Staining with Sudan III—Bone Sectioning and Decalcification—Staining Elastic Tissue—Mounting Unstained Specimens—The Weigert-Pal Method—Golgi Method—Intra Vitam Method—Special Methods for Nervous Tissue................ 11

CHAPTER II.

EXAMINATION OF BLOOD.

Alkalinity — Specific Gravity — Hemaglobin — Tallquist's Method—Gower's Method—Sahli's Method —Dare's Method—Von Fleischl-Meischer Method —The Differential Count—Erythrocytes—Leucocytes—Differential Count of Leucocytes—Use of the Hemacytometer—Blood Smears—Blood Stain—Chenzinsky's Stain—Wright's Method—Staining—Hematoxylin and Eosin—Eosin and Methylene Blue — Ehrlich's Method — Thionin

Malaria—Filaria—Trypanosoma. 21

EXAMINATION OF BODY FLUIDS.

Transudates — Exudates — Cerebro-Spinal Fluid — Cystic Contents — Saliva — Nasal Secretions — Seminal Fluid—Vaginal Secretions—Menstrual Fluids. 34

CHAPTER III.

EXAMINATION OF GASTRIC CONTENTS.

Free Acids—Free Hydrochloric Acid—Congo Red Test — Amidobenzol Test — Boas' Test — Lactic Acid—Uffelman's Test—Strauss' Test - Pepsin and Pepsinogen—Rennin and Renninogen—Blood—Quantitative Tests for Hydrochloric Acid — Organic Acids—Pepsin—Ewald's Test Breakfast—Boas' Test Breakfast—Riegel's Test Dinner —Salzer Test Meal—Ewald-Sievers Method—Gunzburg's Method. 42

EXAMINATION OF FECES.

Reaction—Composition—Blood In—Fatty Acids—Bile—Indol—Phenol—Skatol—Pettenkofer's Test —Pigments—Cholesterin—Microscopic Examination—Intestinal Parasites .56

CHAPTER IV.

THE EXAMINATION OF WATER.

Turbidity—Hazen's Scale—U. S. Geol. Survey Scale —Total Solids—Hardness—Chlorine—Bacteria In —Detection of Sewage—B. Coli.-Mineral Analysis — Coagulation — Precipitation — Filtration — Softening. 65

THE EXAMINATION OF MILK.

Specific Gravity—Total Solids—Ash—Proteids—Casein—Added Water—Fat—Sugar—Bacteria In —Feser's Test—The Babcock Test—Ether Extraction—Scoring of Dairies—Scoring of Milk Depots. 84

CHAPTER V.

URINALYSIS.

The Patient—The Sample—Color—Odor—Transparency — Specific Gravity — Reaction — Total Solids — Approximate and Gravimetric Estimations—Parke's Table—Mineral Ash—Chlorides—Purdy's Method — Purdy's Table — Gravimetric Methods—Titration Methods—Neubauer-Salkowski Method—Salkowski-Volhard Method—Sulfates—Purdy's Method—Purdy's Table—Gravimetric Method—Ehereal Sulfates—Total Sulfur—Phosphates — Purdy's Method — Purdy's Table—Alkaline Phosphates—Earthy Phosphates—Gravimetric Methods—Titration Methods—Albumin—Qualitative Tests—Nitric Acid Contact—Boston's Method—Potassium Ferrocyanide and Acetic Acid — Tanret's Test — Spiegler's Test — Jolles' Test—Quantitative Tests—Centrifugal Methods—Esbach's Method — Purdy's Method — Purdy's Table—Gravimetric Method—Titration Method—Albumin and Globulin — Glucose — Qualitative Tests—Fehling's—Haines'—Quantitative Estimations—Fehling's—Purdy's—Fermentation Method — Differential Density Method — Polarization — Uric Acid — Ruhemann's Method — Ruhemann's Table—Modifications—Folin's Method—Heintz's Method—Purin Bodies—Cook's Method—Urea—Rice's Solutions — Hypobromite Method — The Diazo Reaction. 101

CHAPTER VI.

BACTERIOLOGY.

Preparation of Culture Media—Bouillon—Agar—Gelatin — Milk — Blood Serum — Potato—Ascites Agar—Isolation—Stab Culture—Streak Culture—

Liquefaction—Gas Production—The Gas Formula —Counting Bacteria—Cultivation of Anerobes—Sewage Bacteria—B. Coli.—B. Typhosus—Method of Remy—Method of Hiss—Piorkowski's Method —Method of Adami and Chapin—Drigalski-Conradi Method—Loffler's Malachite Green—Caffein Enrichment Method—The Widal Reaction. Motility, the Hanging Drop Preparation. Staining Bacteria—Common Cover Glass Preparation—The Ziehl Neelson —Method—Gram's Method—Staining Capsules—Buerger's Method—Smith's Method—Staining Flagella—Method of Loffler—Method of Van Ermengem—Staining Spores. Examination of Pus—Method for Gonorrhoea—Examination of Diphtheria-Staining Treponema Pallidum. Animal Inoculation—Post Mortem Bacteriology—The Opsonic Index. 146

APPENDIX.

Table of Atomic Weights. Table of Stains and Reagents. Table of Normal and Decinormal Solutions. 175
Index. 193

CHAPTER I.

HISTOLOGY AND PATHOLOGY.

PREPARATION OF TISSUE.

1 Select desirable pieces of raw tissue.
2 Wash—to remove blood and foreign matter.
3 Cut into small pieces—one-eighth by three-fourths inch.
4 Harden in formalin—5 to 10 per cent.
5 Wash in water.
6 Dehydrate in alcohol—95% to absolute.
7 Place in alcohol and ether, equal parts, few hours to one day.
8 Place in thin celloidin, consistency of oil, one to five days.
    1 Alcohol and ether, equal parts.
    2 Gun cotton or celloidin added to required consistency.
9 Place in thick celloidin, one to five days.
10 Place on tile—one inch circles.
11 Dry till firm in air.
12 Place in jar of 70% alcohol—leave till hard.

PREPARATION OF SECTION.

CUTTING.

1 Place tile with tissue in jaw of microtome.
2 Adjust microtome blade in slanting position.
3 Moisten tissue and float blade with 50% alcohol.
4 Cut sections 10, 15, and 20 microns thick.
5 Remove sections from blade into distilled water.

STAINING.

1 Fill staining dish half full of distilled water.
2 Add 5 to 10 drops of hematoxylin.
3 Transfer sections from distilled water to stain.
4 Cover and let stand some hours—over night is well.
5 Transfer sections to tap water.
6 Place for a moment in eosin.
4 Add drop of Canada balsam.
7 Wash in water.
8 Take section on needle, touch to filter paper, place in alcohol.
9 Again touch to filter paper and place in carbol-cedar oil.

MOUNTING.

1 Take clear section from oil upon spatula.
2 Transfer to center of slide.
3 Blot off excess of oil with filter paper.
5 Place cover glass.

EXPLANATION.

1 Alcohol is used to dehydrate.
2 Distilled water must be used before hematoxylin and to dilute hematoxylin, as hematoxylin and tap water are chemically incompatible.
3 After staining, tap water acts as a mordant to hematoxylin.
4 Some hours in dilute hematoxylin is much better than a short time in strong hematoxylin.
5 Carbol-cedar oil is the best clearing agent. All the essential oils harden and curl sections. The addition of carbolic acid will straighten a curled section and dehydrate 95% alcohol.
6 The steps in the process are consecutive.

A SUGGESTION.

A drop of methylene blue in the tap water after hematoxylin will accentuate cell nuclei.

VAN GIESON'S METHOD.

1 Overstain with hematoxylin.
2 Wash in water.
3 Stain with Van Gieson's picro-fuchsin 3 to 5 minutes.
4 Rinse quickly in water.
5 Dehydrate, clear and mount.

SILVER NITRATE METHOD.

1 Take omentum, peritoneum, or pericardium.
2 Stretch over a board and fasten with tacks.

3 Raise the center with piece of wood to prevent sticking.
4 Wash with solution of silver nitrate..
5 Wash with distilled water.
6 Expose to air and sunlight till dry and brown.
7 Cut out thinnest portions and clear in carbol-cedar oil.
8 Mount in balsam.

THE FREEZING METHOD.

1 Fresh tissue or tissue hardened in formalin.
2 Cut in small, thin pieces.
3 Place on carrier of freezing microtome.
4 Freeze with liquid carbonic acid.
5 Cut sections with plane bit.
6 Stain by ordinary methods..

STAINING FAT.

1 Cut frozen sections.
2 Stain in sudan III.
3 Wash in water.
4 Clear in glycerin and mount in glycerin.

1 Cut frozen sections or take sections of omentum.
2 Stain in solution of osmic acid (.5 to 1%).
3 Wash in water.
4 Dehydrate in ascending alcohols.
5 Clear and mount in glycerin.

BONE SECTIONING.

FIRST METHOD.

1 Select large piece of long bone.
2 Saw off across the shaft.

3 Saw again part through as thin as possible.
4. Withdraw saw and insert thin piece of wood.
5 File the section thin.
6 Cut section off bone and cement to slide with balsam.
7 File till print can be read through section.
8 Remove and clean in xylol.
9 Mount in balsam.

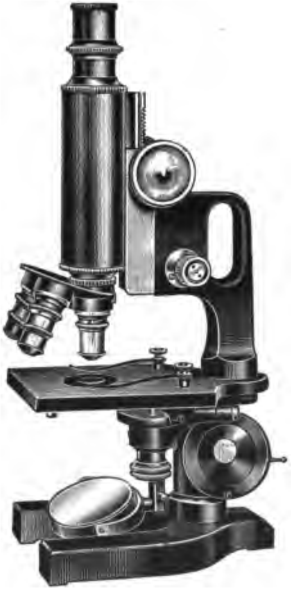


Fig. 1 Compound Microscope (Spencer Lens Co., Buffalo, N. Y.)

SECOND METHOD.

1 Cut fresh bone into pieces of desired size.
2 Fix and harden in formalin.
3 Place in 2% to 5% HNO_3 or HCl.
4 Watch and change solution until decalcified.
5 Wash and dehydrate in alcohol.
6 Place in alcohol and ether equal parts.
7 Infiltrate with celloidin as other tissue.
8 Block, harden, cut, stain and mount as other tissue.

ELASTIC TISSUE.

1 Prepare sections of elastic cartilage or ligamentum nuchae.
2 Stain with a solution of picric acid.
3 Follow with same technique as other sections.

UNSTAINED SECTIONS.

1 Harden eyes in formalin.
2 Open the eye and peel out thin sections of retina and choroid.
3 Dehydrate.
4 Clear in carbol-cedar oil.
5 Mount in balsam.
6 This method gives the best definition of the connective tissue corpuscle, and the hexagonal epithelium of the retina.

WEIGERT-PAL METHOD.

FOR THE BRAIN AND CORD.

THE STAIN.

1 Hematoxylin crystals. . . . . 1 gram.

2	Alcohol 95%	10 c. c.
3	Lithium carbonate, sat. aq. sol.	1 c. c.
4	Water	90 c. c.

THE PAL SOLUTION.

1	Oxalic acid	1 gram.
2	Potassium sulfate	1 gram.
3	Water	200 c. c.

PROCESS.

1 Place sections in 3 to 5% sol. potassium bichromate 24 hours.
2 Wash in water.
3 Place in Weigert stain 24 hours.
4 Wash in water.
5 Place in fresh .25% sol. potassium permanganate one-half to two minutes.
6 Wash in water.
7 Place in the Pal solution one to three minutes.
8 Wash in water several hours.
9 Dehydrate, clear and mount.

THE GOLGI METHOD.

THE MORDANT.

1	1% aq. sol. osmium tetroxid	10 c. c.
2	3.5% aq. sol. potassium bichromate	40 c. c.

SILVER SOLUTION.

1	Crystal $AgNO_3$	.75 gram.
2	Distilled water	100.00 c. c.

PROCESS.

1 Use fresh nervous issue. Small pieces.

2 Place in mordant ten days with frequent changing.
3 Rinse quickly in water.
4 Place in silver solution diluted 1 : 2, 15 minutes.
5 Place in undiluted silver solution 24 to 48 hours.
6 Place in absolute alcohol one hour.
7 Place in equal parts alcohol and ether 30 minutes.
8 Place in thin celloidin 30 minutes.
9 Place in thick celloidin 30 to 45 minutes.
10 Mount on tile.
11 Harden in chloroform 30 min. to 1 hour.
12 Cut at once, moistening knife with oil bergamot.
13 Clear and mount.

INTRA VITAM METHOD.

THE STAIN.

1	Methylene blue.	.1 gram.
2	Distilled water.	100.0 c. c.
3	Dissolve with heat, cool and filter.	

THE FIXING SOLUTION.

1	Ammonium molybdate. . . .	1 gram.
2	Distilled water.	20 c. c.
3	HCl.	1 drop.
4	Make fresh, keep cold, near 0 C.	

PROCESS.

1 Inject blood vessels of living animals with stain, or immerse fresh tissue from living animal in stain.

2 After 10 to 30 min. rinse in normal saline solution.
3 Place in cold fixing sol. 2 to 6 hours. Keep cold.
4 Wash well in distilled water.
5 Dehydrate quickly in 95% to absolute alcohol.
6 Imbed in paraffin.

NISSL'S METHOD.

1	Methylene blue.	3.75	grams.
2	White Castile soap. .	1.75	grams.
3	Distilled water. . . .	1000	c. c.

DIFFERENTIATING SOLUTION.

1	Pure alilin oil.	10 c. c.
2	95% alcohol.	90 c. c.

PROCESS.

1 Fix tissue in 95% to absolute alcohol.
2 Warm stain to steaming.
3 Immerse sections 4 to 6 minutes.
4. Rinse in distilled water.
5 Differentiate in anilin alcohol.
6 Wash in 95% alcohol.
7 Clear in equal parts origanum and cajuput oils.
8 Mount in balsam.

WEIGERT'S ELASTIC TISSUE STAIN.

1 1% aq. sol. basic fuchsin. . . . . 100 c. c.
2 2% aq. sol. resorcin. . . . . 100 c. c.
3 Boil in porcelain dish.
4 Add liquor of $Fe_2 Cl_6$.

5 Heat and stir 5 minutes.
6 Cool and filter.
7 Dry the precipitate.
8 Dissolve in 200 c. c. 95% alcohol.
9 Filter, make up for evaporation, add 4 c. c. HCl.

PROCESS.

1 Stain sections 20 to 60 minutes.
2 Wash, dehydrate, clear and mount.

COMBINED PROCESS.

1 Stain sections in hematoxylin 20 to 30 minutes.
2 Wash in water.
3 Stain in Weigert's stain 20 minutes.
4 Wash in water.
5 Stain in picro-fuchsin 3 to 5 minutes.
6 Rinse quickly in water.
7 Dehydrate, clear and mount.

IMBEDDING IN PARAFFIN.

1 Fix and harden in formalin.
2 Wash in water.
3 Dehydrate in alcohol.
4 Place in chloroform.
5 Melt paraffin—45 to 55 C.
6 Place tissue in paraffin 2 to 6 hours.
7 Pour paraffin into a mould.
8 Place tissue in the mould.
9 Cool till crust is formed.
10 Place in cold water.

CHAPTER II.

EXAMINATION OF BLOOD.

ALKALINITY.

1 Wet red litmus paper with fresh blood.
2 Wash in saline solution.
3 Observe blue color of paper.

ENGEL'S METHOD.

1 Draw .5 c. c. fresh blood in pipette.
2 Mix with 5 c. c. neutral distilled water.
3 Titrate with N/75 tartaric acid.
4 Test reaction after each drop of acid.
5 The tartaric acid contains one gram to the liter.
6. One c. c. N/75 tartaric acid equals .000533 gm. NaOH.
7 No. c. c. tartaric acid used X .000533 X 2 equals alkalinity of one c c. of blood.

SPECIFIC GRAVITY.

1 Use specific gravity apparatus for urine.
2 Mix benzol and chloroform in cylinder to sp. gr. 1.055.

3 Add a drop of fresh blood.
4 Adjust sp. gr. by adding one or the other liquid until drop is suspended midway.
5 Take specific gravity of fluid with urinometer.

TABLES OF SPECIFIC GRAVITY AND HEMAGLOBIN.

HAMMERSCHLAG'S TABLE.

SPECIFIC GRAVITY.	HEMAGLOBIN.
1.033—1.035	25—30%
1.035—1.038	30—35
1.038—1.040	35—40
1.040—1.045	40—45
1.045—1.048	45—55
1.048—1.050	55—65
1.050—1.053	65—70
1.053—1.055	70—75
1.055—1.057	75—85
1.057—1.060	85—95

TABLE OF SCHMALTZ AND PEIPER.

1.030	20%
1.035	30
1.038	35
1.041	40
1.0425	45
1.0455	50
1.048	55
1.049	60
1.051	65
1.052	70
1.0535	75

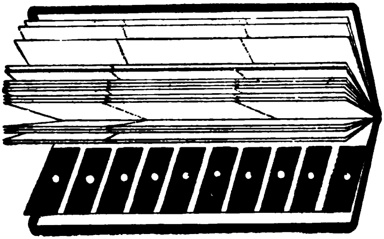
1.056	80
1.0575	90
1.059	100

TOTAL SOLIDS.

1 Place few drops fresh blood on watch crystal.
2. Cover with another watch crystal and weigh at once.
3 Remove cover and dry at 60 to 70 C. 24 hours.
4 Weigh again and calculate percentage.

GUMPRECHT'S TABLE.

Solids,	Men	Women.
Maximum.	23.1%	21.5%
Minimum.	19.6	18.4
Average.	21.6	19.8
Water,		
Average.	78.4	80.2



Fiig. 2. Tallquist Hemaglobin Scale (Edward Pennock, Philadelphia.)

HEMAGLOBIN.

TALLQUIST'S METHOD.

1 Use Tallquist's hemaglobin scale.
2 Draw drop of blood.
3 Saturate piece of the filter paper.
4 Compare with color scale.
5 Read the percentage of hemaglobin.

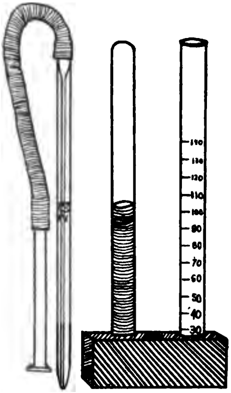


Fig. 3. Gower's Hemaglobinometer.

GOWER'S METHOD.

1 Have Gower's apparatus ready.
2 Draw blood from ear or finger.
3 Fill capillary pipette to mark.
4 Quickly expel into some water in graduated tube.
5 Mix quickly in tube.

6 Add water and compare till same color as standard tube.
7 Read percentage at top of liquid.

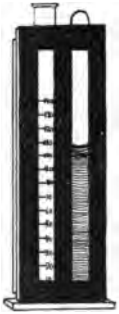


Fig. 4. Sahli's Hemaglobinometer.

SAHLI'S METHOD.

1 Have Sahli's apparatus ready.
2 Fill graduated tube to 10% mark with N/10 HCl. (15 c. c. HCl to 1000 c. c. water.)
3 Fill pipette to mark with blood.
4 Blow and wash quickly into graduated tube and mix.
5 Add water and compare with colored tube.
6 When tints correspond, read percentage at top of fluid.

DARE'S METHOD.

1 Prepare Dare's hemaglobinometer. Light the candle.
2. Draw large drop of blood.

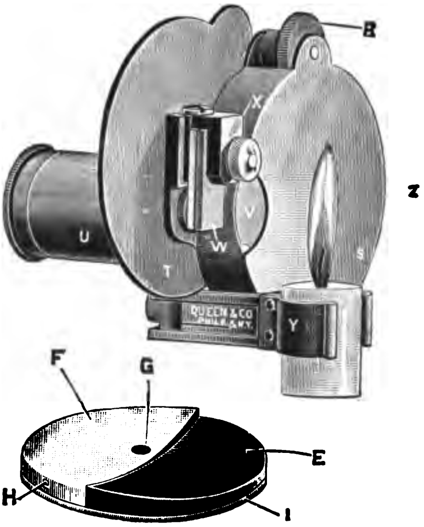


Fig. 5. Dare's Hemaglobinometer (Queen & Co., Philadelphia).

S—Case including prism. T—Movable wing. U—Tube. V—Opening for light. W—White glass of blood-pipette. X—Pipette clamp. Y—Candle holder. E—Colored Glass prism. F—Semi-circle of white glass. H—Index of Hemaglobin. I—Disc of white glass.

3 Fill capillary slit of instrument.
4 Place in the instrument.
5 Adjust the color prism till tints match.
6 Read the percentage.
7 In working, look toward a dark background.

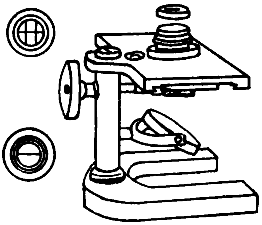


Fig. 6. Von Fleischl-Meischer Hemaglobinometer.

VON FLEISCHL-MEISCHER METHOD.

1 Use the Von Fleischl-Meischer instrument.
2 Prepare a .1% solution of sodium carbonate.
3 Draw a drop of blood from the ear.
4 Fill the pipette for the dilution required.
5 Fill to the 200 mark with the sodium carbonate solution.
6 Mix well by shaking.
7 Expel fluid in capillary tube.
8 Fill the shallow cell with water.
9 Fill the deep cell with the dilute blood.
10 Put on the cover glass and cap.
11 Use in a dark room.
12 Cover with a screen with a slit for the admission of light.
13 Use a yellow light of lamp or candle.

14 Move back and forth with milled head till colors match.
15 Record the reading.
16 Make five to ten readings.
17 Withdraw the fluid and fill the shallow cell.
18 Make readings again.
19 Compare readings, take the mean.
20 Shallow cell is 12 mm. deep cell 15 mm. deep.
21 Their ratio is 4: 5.

THE DIFFERENTIAL COUNT.

NORMAL BLOOD COUNTS.

ERYTHROCYTES.

1 Men 5,000,000 per cubic millimeter.
2 Women 4,500,000 per cubic millimeter.

LEUCOCYTES.

An average of 7,500 per cubic millimeter.

1	Neutrophiles.	70 to 72%
2	Small lymphocytes.	20 to 22%
3	Large lymphocytes. . ..	4 to 8%
4	Eosinophiles.	.5 to 4%
5	Large mononuclears. . ..	1 to 3%
6	Mast cells.	.025 to .5%

TO COUNT ERYTHROCYTES.

1 Use Thoma-Zeiss hemacytometer.
2 Draw drop of blood from ear or finger.
3 Fill short pipette to .5.
4 Quickly draw in saline solution to 101.

5 Mix by shaking and rotating.
6 Expel saline solution from tube below bulb.
7 Place small drop in counting chamber.
8 Place cover glass—drop must cover surface of chamber—must not overflow the moat.
9 Place slide on level stage of microscope.
10 Examine with 1/4, 1/5 or 1/6 objective.
11 Count cells in large number of squares.
12 Take average.
13 Multiply by 4000 and by 200.

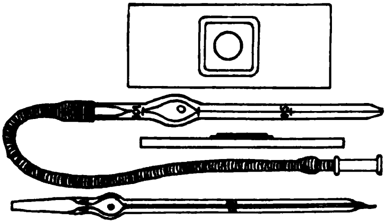


Fig. 7. Thoma-Zeiss Hemacytometer.

TO COUNT LEUCOCYTES.

1 Draw drop of blood.
2 Fill long pipette to .5.
3 Draw in dilute acetic acid to 11.
4 Mix.
5 Expel acid in tube below bulb.
6 Place drop in counting chamber and cover.
7 Place slide on microscope.
8 Count leucocytes in large number of squares.

9 Take average.
10 Multiply by 4000 and by 20.

DIFFERENTIAL COUNT OF LEUCOCYTES.

1 Make blood smears.
2 Stain by Wright's method.
3 Make a chart of leucocytes thus:—
    1 Neutrophiles.
    2 Small lymphocytes.
    3 Large lymphocytes.
    4 Eosinophiles.
    5 Large mononuclears.
    6 Mast cells.
    7 Myelocytes.
    8 Eosinophile myelocytes.
4 Count 200 to 400 leucocytes and tally each kind.
5 Estimate percentage of each.

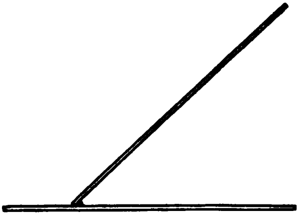


Fig. 8. The Blood Smear with Slides.

BLOOD SMEARS.

FIRST METHOD.

1 Prepare a number of clean slides.
2. Draw a large drop of blood.

3 Take up drop on end of a slide.
4 Apply to another slide at an angle.
5 Quickly draw first slide along second.
6 Repeat the process quickly with the other slides.
7 Coagulation spoils the smears.

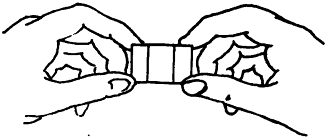


Fig. 9. Smear With Cover Glasses.

SECOND METHOD.

1 Place drop of blood on cover glass.
2 Cover quickly with another cover glass.
3 Draw the two cover glasses apart.

THIRD METHOD.

1 Draw a drop of blood.
2 Take up on end of piece of cigarette paper.
3 Apply to slide or cover glass.
4 Draw paper along the glass.

STAINING BLOOD.

HEMATOXYLIN AND EOSIN.

1 Make blood smears on slides.
2 Fix smears in flame.
3 Stand in staining jar of hematoxylin, 10 minutes or more.
4 Wash in tap water.

5 Stand in staining jar of eosin short time.
6 Wash, dry and mount.

EOSIN AND METHYLENE BLUE.

1 Make blood smears on slides, fix in flame.
2 Stand in staining jar of eosin.
3 Wash in water.
4 Place in staining jar of methylene blue.
5 Wash in water.
6 Dry and mount.

EHRLICH'S METHOD.

1 Make blood smears on slides.
2 Fix in flame.
3 Stain with Ehrlich's triple stain.
4 Wash in water.
5 Dry and mount.

WRIGHT'S METHOD.

1 Make smears on slides.
2 Dry in air.
3 Cover with Wright's stain 1 to 2 minutes.
4 Add water till metallic luster appears—2 min.
5 Wash in water till smear appears pink.
6 Blot off with filter paper.
7 Dry and mount.
8 This is the best method for blood.

THIONIN STAIN.

THE STAIN.

1 Sat. sol. thionin in 50% alcohol.... 20 c. c.

2 2% carbolic acid. . . . . .100 c. c.
3 Mix and let stand some days.

THE PROCESS.

1 Fix smears in alcohol-formalin.
2 Stain 10 to 15 seconds.
3 Wash, dry and mount.
4 Particularly valuable in malaria.

CHENZINSKY'S METHOD.

THE STAIN.

1	Methylene blue, sat. aq. sol.	40 c. c.
2	Eosin, .5% sol. in 70% alcohol	20 c. c.
3	Distilled water	40 c. c.

PROCESS.

1 Fix smears in alcohol.
2 Place in staining jar of stain.
3 Place in incubator at 37 C. 3 to 6 hours.
4 Wash, dry and mount.

FILARIA.

1 Take a large drop of fresh blood on slide.
2 Cover with cover glass.
3 Examine at once with microscope.
4 Filaria can be detected by commotion among blood cells.

TRYPANOSOMA.

1 Make smears from lymph glands, or
2 From fluid of edematous areas.
3 Stain by Wright's method.

RED BONE MARROW AND SPLENIC PULP.

1 Make smears on slides.
2 Stain by same methods as blood.

EXAMINATION OF BODY FLUIDS.

TRANSUDATES.

1 Generally serous.
2 Generally straw colored.
3 Sometimes mixed with blood.
4 Generally of low specific gravity.
    1 1.005 to 1.015.
    2 Pleural inflammatory 1.018 to 1.030.

SPECIFIC GRAVITY.

Use the method for urine.

ALBUMIN.

Use the methods for urine.

RUESS' TABLE.

ALBUMIN IN TRANSUDATES AND EXUDATES.

Specific Gravity.	Albumin.
1.008	.2%
1.009	.6
1.010	1.0
1.011	1.3
1.012	1.7
1.013	2.1
1.014	2.5
1.015	2.8
1.016	3.2
1.017	3.6

1.018	4.0
1.019	4.3
1.020	4.7
1.021	5.1
1.022	5.5
1.023	5.8
1.024	6.2
1.025	6.6
1.026	7.0
1.027	7.3
1.028	7.7

MICROSCOPIC EXAMINATION.

1 Collect by aspiration.
2 Inoculate agar tubes and Petri plates.
3 Collect the sediments.
    1 Place a portion in centrifuge tube.
    2 Centrifugate.
    3 Decant fluid.
    4 Take up sediment in pipette.
    5 Place drop on end of slide.
    6 Make a smear as in examination of blood.
    7 Stain by various methods for bacteria.
    8 Filter another portion through small fine filter paper.
    9 Take up last few drops in pipette.
    10 Make smears as above.
    11 Make common cover glass preparation.
    12 Stain by Ziehl-Neelson method for T. B.

EXUDATES.

SEROUS EXUDATES.

1 Clear, pale, or light straw colored serum.
2 Specific gravity not above 1.008.
3 Make smears and cover glass preparations.

INOSCOPY.

METHOD OF JOUSSET.

1 Digest with following fluid:

Pepsin	2 grams
Glycerin	10 c. c.
HCl	10 c. c.
Sodium fluorid	3 grams
Distilled water	1000 c. c.

2 Centrifugate.
3 Stain sediment for T. B.
4 Inoculate guinea pigs.
5 Examine guinea pigs for tuberculosis.

CHYLOUS EXUDATES.

1 Examine for sugar as in urine.
2 Stain slides with sudan III for fat.

HEMORRHAGIC EXUDATES.

1 Make smears of the sediments.
2 Stain by the methods for blood.

PURULENT EXUDATES.

1 Make cover glass preparations of the sediment.
2 Stain for T. B.
3 Stain for the other bacteria by methods given under bacteriology.

CEREBRO-SPINAL FLUID.

COLLECTION.

1 From anterior fontanelle in early life.
2 From second lumbar interspace.

CULTURES.

1 Make cultures on blood serum, agar-agar and glycerine agar, and in bouillon.
2 Incubate at 37 C. 24 to 48 hours.

STAINING.

1 When collecting, let drops fall on clean slides.
2 Dry in air.
3 Fix in flame.
4 Stain by following methods:—
    1 Ziehl-Neelson for T. B.
    2 Gram's method for diplococcus intracellularis.
    3 Common cover glass preparation with methylene blue.

SYNOVIAL FLUID.

1 Collect with hypodermic needle.
2 Stain for bacteria by methods given above.
3 Make cultures and examine.

THE SALIVA.

COLLECTION.

1 Wash the mouth.
2 Expectorate into a filter till the proper amount is collected.

3 Collect normal saliva in a separate vessel.
Quantity—200 to 1500 c. c. in 24 hours.
Specific gravity—1.002 to 1.006.
Reaction—Alkaline.
Determined as in urine.

DIASTASIC FERMENT.

1 5 c. c. filtered saliva.
2 50 c. c. starch water.
3 Heat over water bath at 40 C. for 1 hour.
4 Test for sugar by methods for urine.

COMPARATIVE DIGESTING POWER.

1 Prepare a normal filtered saliva.
2 Prepare filtered saliva to be tested.
3 Prepare boiled starch water.
4 Place 10 c. c. starch water in 12 test tubes.
5 Add 2 c. c. normal saliva to 6 tubes.
6 Add 2 c. c. saliva to be tested to other 6 tubes.
7 Incubate at 40 C.
8 Test one tube of each set with a drop of iodin solution every 2 minutes.
9 The iodin reaction should fail at 10 minutes with normal saliva.
10 Compare with time of sample under examination.

NASAL SECRETION.

Saline taste.
Alkaline reaction.

MICROSCOPIC EXAMINATION.

1 Make smears on slides or cover glasses.

2 Use Ziehl-Neelson method for T. B.
3 Use Gram's method in meningitis.
4 Pneumococcus may be found.
5 Bacillus of influenza early in the disease.

PUS IN THE EYE.

1 Examine for gonococcus.
    1 Common cover glass preparation.
    2 Gram's method.
2 Stain for
    1 Staphylococcus pyogenes aureus.
    2 Streptococcus pyogenes.
    3 Bacillus of diphtheria.
    4 Morax-Axenfeld diplobacillus.
    5 Koch-Weeks bacillus.

THE SEMINAL FLUID.

1 Collect in small bottle.
2 Keep well corked.
3 Keep at 37 C.

MOTILITY.

1 Warm a slide to body temperature.
2 Place a drop of semen on the center of slide.
3 Place a warm cover glass.
4 Examine with 1/6 objective.

STAINING.

1 Spread semen on cover glass or slide.
2 Dry and fix in the flame.
3 Stain with methylene blue or carbol fuchsin.

VAGINAL SECRETION.

1 Normally small in amount.
2 Mucus, serves as a lubricant.
3 Contains desquamated epithelium.
4 Acid in reaction.

EXAMINATION.

1 Make smears on slides or cover glasses.
2 Dry in air.
3 Fix in flame.
4 Stain by the various methods for bacteria.
5 The smegma bacillus is acid fast.

GONORRHOEA IN THE FEMALE.

1 Collect the pus from the urethra.
2 Make smears on slides or cover glasses.
3 Dry in air.
4 Fix in flame.
5 Stain by ordinary methods and by Gram.

DIPHTHERIA OF VULVA.

1 Remove portions of the membrane.
2 Make smears on slides or cover glasses.
3 Dry and fix.
4 Stain with Loeffler's methylene blue.
5 Make a number of agar slant cultures.
6 Incubate at 37 C. 6, 12, and 24 hours.
7 Make common cover glass preparations.
8 Examine with oil immersion lens.

A wet preparation of vaginal secretion may contain Trichomonas vaginalis, oxyuris and its ova, and spermatozoa.

MENSTRUAL FLUID

1 Make smears as blood smears.
2 Fix and stain as in technique for blood.

ABORTION.

1 Make smears.
2 Stain with hematoxylin and eosin.
3 Examine for chorionic villi.
4 Examine for decidual cells.

CHAPTER III.

EXAMINATION OF GASTRIC CONTENTS.

QUALITATIVE TESTS.

FREE ACIDS.

CONGO-RED TEST.

1 Test tube two thirds full of distilled water.
2 Add Congo-red to a strong red color.
3 Add filtered gastric juice drop by drop.
4 A blue color indicates free acid.
5 A marked blue reaction indicates free HCl.

FREE HCL.

AMIDO-BENZOL TEST.

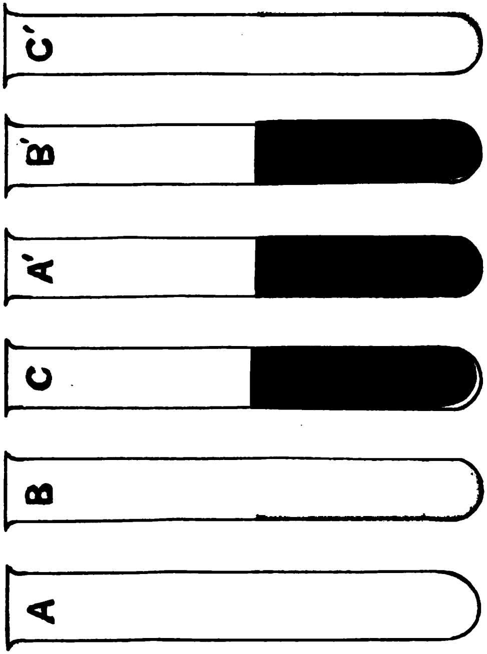
1 One-half to one c. c. filtered gastric juice in a tt. or porcelain dish.
2 Add one drop of 5% alcoholic sol. dimethyl amido-azobenzol.
3 A cherry red color indicates HC1.
4 Organic acids above .5% give same reaction.

BOAS' RESORCIN TEST.

REAGENT.

1 5 grams resublimed resorcin.

PLATE II.



A, Gastric fluid to which a 1 per cent. solution of phenolphthalein has been added; B, gastric fluid to which a 1 per cent. solution of alizarin has been added; C. gastric fluid to which a 0.5 per cent. solution of dimethylamido-azobenzol has been added; A', A after titration with a decinormal solution of sodium hydroxid; B', B after titration with a decinormal solution of sodium hydroxid; C', C after titration with a decinormal solution of sodium hydroxid (Boston).

2 3 grams cane sugar.
3 100 c. c. alcohol.

PROCESS.

1 Place few drops filtered gastric juice in porcelain dish.
2 Add few drops of reagent.
3 Slowly evaporate to dryness without scorching.
4 A rose red color identifies HCl.
5 This color fades on cooling.

PHLOROGLUCIN-VANILLIN TEST.

REAGENT.

1 2 grams of phloroglucin in a mortar.
2 Add 1 gram of vanillin.
3 Reduce to powder and dissolve in 100 c. c. 80% alcohol.
4 Keep in colored glass stoppered bottle.

PROCESS.

1 Place few c. c. filtered gastric juice in t.t.
2 Add equal amount of reagent.
3 Heat very gently, do not boil or evaporate.
4 Fine rose colored lines indicate .05% HCl. or more.

TROPAEOLIN TEST.

1 4 or 5 drops sat. alcoholic sol. tropaeolin 00 in porcelain dish.
2 Grasp dish in forceps and tilt from side to side to spread fluid.
3 Add equal amount of gastric juice.

4 Tilt the dish and mix.
5 Heat very gently over Bunsen burner.
6 Lilac blue lines indicate .2 to .3% HCl.

MOHR'S TEST.

REAGENT.

1 2 c. c. 10% solution of KSCN.
2 .5 c. c. neutral solution ferric acetate.
3 10 c. c. distilled water.

PROCESS.

1 2 to 5 drops of reagent on one side of porcelain dish.
2 Equal amount filtered gastric juice on other side.
3 Permit them to run together slowly.
4 Light violet color at contact indicates free HCl.
5 Deep mahogany color when well mixed.

LACTIC ACID.

UFFELMANN'S TEST.

1 5 c. c. filtered gastric juice in tt.
2 Add 50 c. c. ether and shake 10 minutes.
3 Decant the ether.
4 Evaporate the ether over water bath.
5 Dissolve residue in 5 c. c. water and test as follows:—
    1 Place three drops phenol in tt.
    2 Add three drops sat. aq. sol. ferric chlorid.
    3 Mix and add water to an amethyst blue color.

4 Pour in the gastric solution.
5 A canary yellow color indicates lactic acid.

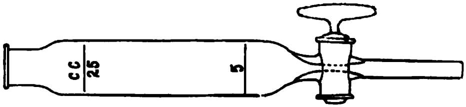


Fig. 10. Strauss' Separatory Funnel.

STRAUSS' TEST.

1 Use Strauss' separatory funnel.
2 Fill to 5 c. c. with filtered gastric juice,
3 And to the 25 c. c. mark with ether.
4 Shake ten or 15 minutes.
5 Let stand till the ether separates.
6 Draw off to the 5 c. c. mark. Fill to 25 c. c. with water.
7 Add 2 drops tinct. ferric chlorid diluted 1 to 10.
8 Shake gently.
9 A strong yellowish green color indicates .1% or more.
10 A pale green will appear with .05%.

KELLING'S TEST.

1 One c. c. filtered gastric juice in tt.
2 Add 9 c. c. water.
3 Hold up to the light and add
4 One to 3 drops 5% sesquichlorid of iron.
5 A greenish yellow color indicates lactic acid.

BOAS REACTION.

1 To detect acetic aldehyd.

2 Make up Nessler's solution.
  1 Dissolve 2 grams KI in 50 c. c. water.
  2 Add iodid of mercury in variable amount.
  3 Heat until only a portion of the mercury remains.
  4 Cool and add 20 c. c. water.
  5 Place in glass stoppered bottle.

  1 Take 2 parts of this solution, and
  2 Three parts concentrated KOH.
  3 Mix, let stand, filter, place in glass stoppered bottle.

PROCESS.

1 Test for free acids by Congo red test.
2 In presence of free acids, add excess of barium carbonate to gastric juice and filter.
3 Take 10 c. c. of filtrate.
4 Evaporate to syrup over water bath.
5 Add few drops phosphoric acid and boil.
6 Cool, and add 100 c. c. ether.
7 Shake at intervals for an hour.
8 Decant the ether and evaporate.
9 Dissolve residue in 45 c. c. water.
10 Shake thoroughly and filter.
11 Add 5 c. c. H_2SO_4 and small amount MnO_2.
12 Place in Erlenmeyer flask.
13 Connect with cylinder containing 10 c. c. Nessler's solution.
14 Gradually heat to boiling.
15 Positive reaction is a yellowish red precipitate, aldehyd of mercury.
16 If iodopotassic iodid be used instead of Nes-

sler's solution, the precipitate is iodoform.

KNAPP'S METHOD.

1 One c. c. filtered gastric juice.
2 5 c. c. ether.
3 Place both in tt. and shake.
4 Place about 2 c. c. 1 : 2000 ferric chlorid sol. in t.t.
5 Run in ether extract slowly on top.
6 A canary colored ring indicates lactic acid.

DE JONG'S METHOD.

1 5 c. c. filtered gastric juice.
2 Add one or two drops HCl.
3 Evaporate to syrupy consistence.
4 Extract with ether.
5 Make up to 5 c. c. with water.
6 Add one drop 5% sol. Fe_2Cl_6 and shake.
7 .05% lactic acid give definite green color.

PEPSIN AND PEPSINOGEN.

1 Boil an egg hard and peel off the shell.
2 Cut cylinders from the white with cork borer.
3 Cut the cylinders into small discs.
4 Number three test tubes, 1, 2, 3.
5 Place a disc in each.
6 In No. 1, place 10 c. c. distilled water, .3 gram pepsin, and 3 drops official dilute HCl.
7 In No. 2, place 10 c. c. filtered gastric juice.

8 In No. 3, place 10 c. c. filtered gastric juice, and 3 drops official dilute HCl.
9 Incubate at 37 C. 3 hours, then examine at intervals.
10 No. 1 should show normal digestion.
11 Digestion in No. 2 indicates both pepsin and free HCl.
12 Failure in No. 2, and digestion in No. 3, indicates presence of pepsinogen.
13 Failure in No. 3 indicates absence of pepsin and pepsinogen.

RENNIN.

1 5 c. c. filtered gastric juice in tt.
2 Neutralize with very dilute NaOH.
3 Add 5 c. c. fresh milk.
4 Incubate at about 40 C.
5 Milk should coaguluate in 10 to 15 minutes.

RENNINOGEN.

1 5 c. c. filtered gastric juice in tt.
2 Neutralize with very dilute NaOH.
3 Add 2 c. c. 1% sol. calcium chlorid.
4 Incubate.
5 If renninogen is present, milk will coagulate.

BUTYRIC ACID.

1 10 c. c. filtered gastric juice.
2 Extract with 50 c. c. of ether.
3 Decant and evaporate the ether.
4 Dissolve residue in few c. c. of water.
5 Add a trace of powdered calcium chlorid.

6 Butyric acid will separate as oil droplets.
7 Butyric acid has the odor of rancid butter.

ACETIC ACID.

1 10 c. c. filtered gastric juice.
2 Extract with ether.
3 Evaporate the ether to dryness.
4 Dissolve residue in small amount of water.
5 Neutralize with weak NaOH.
6 Add 1 to 3 drops very weak sol. ferric chlorid.
7 Dark red color indicates acetic acid.

QUANTITATIVE ESTIMATIONS.

TOTAL ACIDITY.

TOPFER'S METHOD.

1 10 c. c. filtered gastric juice in evaporating dish.
2 Add 3 or 4 drops 1% alcoholic sol. phenolphthalein.
3 Titrate with decinormal NaOH.
4 Multiply c. c. NaOH used by 10 for degrees of acidity.
5 Multiply degrees of acidity by .00365 for per cent HCl.

FREE HCL.

1 10 c. c. filtered gastric juice in beaker.
2 Add 3 or 4 drops .5% alcoholic sol. dimethyl-amido-azobenzol.
3 Titrate with decinormal NaOH.
4 Calculate to degrees and HCl as above.

5 For other acidities, add 3 or 4 drops phenolphthalein.
6 Continue the titration.
7 Estimate as before.

COMBINED HCL.

1 10 c. c. filtered gastric juice in beaker.
2 Add 4 drops 1% aq. sol. sodium alizarin sulphonate.
3 Titrate with decinormal NaOH.
4 End point is a bluish-violet color.
5 Multiply c. c. NaOH used by 10.
6 This gives degrees of acidity less combined HCl.
7 Subtract from total acidity.

ORGANIC ACIDS.

1 Determine total acidity in one portion of gastric juice.
2 Thoroughly extract another portion with ether.
3 Separate the ether.
4 Estimate the total acidity of the residue.
5 The difference is organic acids.

HEHNER-SEEMANN METHOD.

1 20 c. c. filtered gastric juice in beaker.
2 Add 3 or 4 drops phenolphthalein.
3 Neutralize with decinormel NaOH.
4 Evaporate to dryness and incinerate.
5 Do not burn after radiant flame ceases.
6 Dissolve ash in water.

7 Titrate with decinormal HCl, using phenolphthalein again as an indicator.
8 Multiply c. c. HCl used by .00365.
9 Result is organic acids in terms of HCl.

FATTY ACIDS.

CAHN-MEHRING-MCNAUGHT METHOD.

1 Determine total acidity in one portion of 10 c. c.
2 Evaporate another 10 c. c. to syrupy consistence.
3 Dilute with water.
4 Determine total acidity as before.
5 Take the difference for amount of fatty acids.

LACTIC ACID.

REAGENTS.

1 Decinormal iodin, 12.697 grams to liter of water.
2 Decinormal sodium thiosulphate, 24.8 grams to liter.
3 Solution KOH, 56 grams to liter.
4 HCl, specific gravity 1.018.
5 Starch solution.
    1 Dissolve 5 gm. starch in 100 c. c. warm water.
    2 Dissolve 10 gm. $ZnCl_2$ in 100 c. c. water.
    3 Add to warm starch solution.

PROCESS.

1 Test for free acid by Congo red test.
2 In presence of free acids, add excess of $BaCO_3$ to 10 c. c. gastric juice and filter.
3 Evaporate to syrup over water bath.
4 Add few drops phosphoric acid and boil.
5 Cool, and add 100 c. c. ether.
6 Shake at intervals for an hour.
7 Decant the ether and evaporate.
8 Dissolve residue in 45 c. c. water.
9 Shake thoroughly and filter.
10 Add 5 c. c. H_2SO_4 and a small amount of MnO_2.
11 Place in flask with two tubes.
12 Connect one tube to rubber tube with clamp and bulb.
13 Connect other tube to distilling apparatus.
14 Distil with low heat .75 to .8 of the mixture.
15 Mix 20 c. c. decinormal iodin and 20 c. c. KOH, and add to distillate.
16 Shake well and stand ten minutes.
17 Add 20 c. c. HCl.
18 Titrate with decinormal thiosulphate.
19 Near the end add a little starch solution and finish the titration.
20 20 c. c. iodin — number c. c. thiosulphate used indicates iodin used to form iodoform.
21 One c. c. decinormal iodin equals .003388 gram lactic acid.

22 .003388 X c. c. iodin used X 10 equals per cent of lactic acid.

PEPSIN.

1 Take the white of an egg.
2 Add 12 volumes of .4% HCl.
3 Mix well and filter.
4 This solution will be 1% egg albumin.
5 Take 10 c. c. in each of three tt.
6 To No. 1, add 5 c. c. filtered gastric juice.
7 To No. 2, add 5 c. c. water and .5 gram pepsin.
8 To No. 3, add 5 c. c. water.
9 Place in incubator at 37 C. for an hour.
10 Determine the albumin in each tube.
11 Difference between 2 and 3 is normal digestion.
12 Difference between 1 and 3 is digestion of the sample.
13 Amount of pepsin varies as the square of the digestion.
14 Therefore, if No. 1 : No. 2 : : 2 : 4, then pepsin is 4 : 16.

BLOOD IN STOMACH CONTENTS.

1 Take 10 c. c. stomach contents.
2 Add few c. c. glacial acetic acid.
3 Add equal volume of ether.
4 Shake and mix thoroughly.
5 Separate the ether.
6 Test the ether by the guiac method. (See urine.)

EWALD'S TEST BREAKFAST.

1 One roll or two slices of bread without butter.
2 300 to 400 c. c. water or weak tea without cream or sugar.
3 Well masticated.
4 Stomach contents removed after one hour.

BOAS' TEST BREAKFAST.

1 One tablespoonful of rolled oats.
2 One liter of water.
3 Boil to 500 c. c.
4 Add a pinch of salt.
5 Remove from stomach in 45 min. to one hour.

REIGEL'S TEST DINNER.

1 400 c. c. soup.
2 200 grams beef steak chopped fine.
3 50 grams wheat bread.
4 200 c. c. water.
5 Remove stomach contents 4 hours after meal.

THE SALZER TEST MEAL.

BREAKFAST.

1 30 grams lean, cold, roast beef chopped fine.
2 250 c. c. milk.
3 50 grams of rice.
4 One soft boiled egg.

1 After four hours give an Ewald or Boas test breakfast.
2 Remove stomach contents one hour later.

MOTOR POWER OF THE STOMACH.

LEUBE'S METHOD.

1 Wash the stomach clean.
2 Give Riegel's test dinner.
3 Remove in six hours.
4 Wash stomach with 300 c. c. warm water.
5 Repeat washing two or three times till water is clear.
6 If only minute particles of food are found,

motor power is normal.

EWALD-SIEVERS' METHOD.

1 Give a 1 gram capsule of salol after full meal.
2 Collect separate bottles of urine after 30 min., 1 hour, 1.5 hour, 2, 12, and 24 hours.
    1 Place few c. c. in test tube.
    2 Add small amount of Fe_2Cl_6.
    3 Reaction is a violet color.
4 Positive reaction should appear in 45 to 75 minutes.
5 The 24 hour sample should be negative.

GUNZBURG'S METHOD.

1 Give Ewald's test breakfast.
2 Give Gunzburg's packet 45 minutes later.
    1 .2 to .3 gram KI.

   2 Place in thin rubber tube tied with fibrin.
3 Examine the saliva for iodin every 15 minutes after one hour.

CHEMISTRY OF GASTRIC CONTENTS.

Water	904.4
Solids	5.6
Organic matter	3.19
NaCl	1.46
$CaCl_2$	.06
KCl	.55
HCl	.2
Phosphate of iron and magnesium	.12

SENSITIVENESS OF REAGENTS TO HCL.

1	Dimethyl-amido-azobenzol	.02	pro mille
2	Phloroglucin-vanillin	.05	"
3	Resorcin	.05	"
4	Tropaeolin 00	.3	"
5	Mohr's reagent	1.00	"

EXAMINATION OF FECES.

MACROSCOPIC.

1 Quantity—100 to 150 gm. in 24 hours.
2 Frequency.
3 Form.
4 Consistency.
5 Color.
6 Odor.

7 Mucus.
8 Concretions.
9 Animal parasites.

CONCRETIONS AND FOREIGN BODIES.

1 Catch the whole stool in a vessel.
2 Place in a sieve of about 20 mesh.
3 Add water.
4 Use a brush to work up the feces.
5 Pass all the soft materials through the sieve.
6 Gall stones and foreign bodies will remain.

ANIMAL PARASITES.

1 Give a vermicide and laxative.
2 Collect the whole stool.
3 Proceed as for concretions.

CHEMICAL EXAMINATION.

BLOOD.

1 Exclude iron from the medicines.
2 Exclude blood from the food.
3 Diet of bread, milk, eggs and fruit.
4 Give large dose of powdered charcoal.
5 Examine stools after charcoal has passed.
6 Soften portion of stool with water.
7 Treat with one-third its volume of glacial acetic acid.
8 Extract with ether and add a little alcohol.
9 Separate the ether and apply the gum guiac test.

BILE—BILIRUBIN.

1 Add water to consistency for filtration.

2 Filter.
3 Apply Gmelin's test, as for urine.

REACTION.

1 Normally alkaline due to ammoniacal fermentation.
2 Sometimes neutral.
3 Sometimes acid from lactic or butyric acid.

COMPOSITION OF NORMAL FECES.

Water	77.3%
Mucin	2.3
Proteids	5.4
Extractives	1.8
Fats	1.5
Salts	1.8
Resinous, biliary, and coloring matters	5.2
Residue of food	4.7

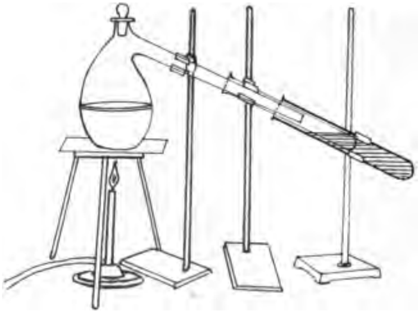


Fig. 11. A Simple Still.

PHENOL, INDOL, SKATOL.

1 Dilute feces with water.
2 Acidify with phosphoric acid.
3 Distil.
4 Neutralize distillate with sodium carbonate.
5 Distil again.
6 Make distillate alkaline with KOH.
7 Distil again. Preserve distillate. Contains indol and skatol.
8 Acidify residue in retort with H_2SO_4.
9 Distil again.
10 Test distillate for phenol.
  1 Place portion in tt.
  2 Add few drops Fe_2Cl_6.
  3 An amethyst blue indicates phenol.
  1 Place another portion in tt.
  2 Add Millon's reagent.
    1 Mercury. . . . . .1 part.
    2 HNO_3. . . . . .2 "
    3 Water. . . . . .6 "
  3 A red color indicates phenol.

INDOL.

1 Take the distillate of No. 7 above.
2 Cool to deposit indol and skatol.
3 Add warm water and carefully bring to 52 C.
4 Maintain this temperature and filter.
5 Test filtrate for indol.
  1 Add nitric acid.
  2 Add sodium nitrate.
  3 A red precipitate indicates indol.

SKATOL.

1 Take the residue from No. 4 under indol.
2 Add Nitric acid.
3 Add sodium nitrate.
4 This gives a milky mixture.

FATTY ACIDS.

1 Dilute feces with water.
2 Acidify with phosphoric acid.
3 Distil.
4 Neutralize distillate with sodium carbonate.
5 Distil a second time.
6 Evaporate residue to dryness on water bath.
7 Extract residue with alcohol.
8 Evaporate the alcohol.
9 Dissolve the residue in water.

FORMIC ACID.

1 Distil last sol. above at 100 C.
2 Test distillate for formic acid.
    1 Concentrate the distillate.
    2 Add NaOH or KOH.
    3 Add $AgNO_3$.
    4 Apply heat, a black precipitate is formed.

ACETIC ACID.

1 Continue distillation of same sol. at 119 C.
2 Test distillate for acetic acid.
    1 Take the odor.
    2 Neutralize cautiously with sodium carbonate.

3 Add a few drops Fe_2Cl_6.
4 A blood red color results.

BUTYRIC ACID.

1 Continue distillation of same sol. at 137 C.
Has the odor of rancid butter.
Add acid and take the odor.

VALERIANIC ACID.

1 Continue distillation of same sol. at 176.3 C.
2 Add sol. $AgNO_3$ to distillate.
3 Crystals in plates are formed.
4 Crystals not readily soluble.

BILE ACIDS.

1 Dilute feces with water.
2 Acidify with phosphoric acid.
3 Distil.
4 Neutralize distillate with sodium carbonate.
5 Distil again.
6 Make distillate alkaline with KOH.
7 Distil again.
8 Acidify residue in retort with H_2SO_4.
9 Distil again.
10 Dissolve residue in water.
11 Boil and filter.
12 Treat with PbAc, add small amount NH_4OH.
13 A precipitate of lead salts is formed.
14 Wash precipitate with water.
15 Boil with alcohol.
16 Filter.

17 Treat filtrate with sodium carbonate.
18 Filter again.
19 Evaporate filtrate to dryness.
20 Extract with hot alcohol.
21 Treat the precipitate with ether.

PETTENKOFER'S TEST.

1 Place small amount of precipitate in a porcelain dish.
2 Dissolve in distilled water.
3 Add two-thirds its volume concentrate H_2SO_4.
4 Maintain temperature not above 70 C.
5 Add 10% sol. saccharose drop by drop.
6 First a beautiful red color, changing to violet on standing.

CHOLESTERIN.

1 Separate fatty acids, phenol, indol and skatol by distillation.
2 Add H_2SO_4 to residue.
3 Extract with alcohol. Preserve the extract.
4 Extract with ether.
5 Filter the ether.
6 Distil off the ether.
7 Treat the residue with sodium carbonate.
8 Evaporate to dryness and extract with ether.
9 Filter the alcoholic extract.
10 Add excess of sodium carbonate.
11 Distil off the alcohol.
12 Extract with ether.
13 Place ether in beaker and evaporate over water bath.

14 Add alcoholic sol. KOH.
15 Evaporate over water bath, dilute with water and extract with ether.
16 Separate the ether and examine with the microscope.
17 Place crystals of cholesterin in tt.
18 Add chloroform.
19 Add H_2SO_4.
20 Shake one-half minute.
21 Chloroform will become red, sulfuric acid fluorescent.

MICROSCOPIC EXAMINATION.

1 Select portions of feces from macroscopic examination.
2 Work up with distilled water.
3 Place drop on center of slide.
4 Place cover glass.
5 Examine with a two-thirds objective.
6 Study particular objects with a 1/6 to 1/8 objective.
7 Touch a drop of iodin solution to edge of cover glass. Starch will turn blue.
8 Stain a slide with osmic acid or sudan III for fat.
9 The wet preparation may also show:
    1 Particles of undigested food.
    2 Vegetable spirals.
    3 Vegetable cells.
    4 Epithelial cells.
    5 Crystals.

        1. Of fatty acids.
        2 Charcot-Leyden.
        3 Cholesterin.
        4 Hematoidin.
        5 Phosphates.
        6 Hemin.
        7 Bismuth.
    6 Animal parasites and their ova.
        1 Ameba coli.
        2 Trichomonas.
        3 Cercomonas intestinalis.
        4 Balantidium coli.
        5 Tape worms.
            1 Taenia solium.
            2 Taenia mediocanellata.
            3 Dibothriocephalus latus.
            4 Taenia echinococcus.
        6 Trematodes or flukes.
        7 Round worms.
            1 Ascaris lumbricoides.
            2 Oxyuris vermicularis.
            3 Uncinaria.
                1 Duodenalis.
                2 Americana.
            4 Trichocephalus dispar.
            5 Strongyloides intestinalis.
            6 Strongyloides substilis.
            7 Anguillula intestinalis.
            8 Trichina spiralis.

CHAPTER IV.

THE EXAMINATION OF WATER.

TURBIDITY.

THE METHODS ARE PHOTOMETRIC.

METHOD WITH TURBIDITY RODS.

1 Use Hazen's turbidity rod, or
2 Use U. S. Geol. Survey revised rod of 1902.
3 Draw water into large container.
4 Examine in open light but not in sunlight.
5 Insert rod in water till wire just disappears.
6 Read turbidity on rod at top of wet portion.
7 Use same method in tanks, reservoirs, settling basins, streams and lakes.

GRADUATION OF HAZEN'S SCALE.

Distance from wire. Inches.	Turbidity. Reciprocal.
.25	4.00
.5	2.00
.75	1.33
1.00	1.00

1.5	.66
2.00	.5
2.5	.4
3.0	.33
3.5	.285
4.0	.25
4.5	.22
5.0	.2
5.5	.18
6.0	.166
6.5	.153
7.0	.142
7.5	.133
8.0	.125
8.5	.117
9.0	.111
9.5	.105
10.0	.1
11.0	.09
12.0	.083
13.0	.076
14.0	.071
15.0	.066
16.0	.0625
17.0	.0588
18.0	.055
19.0	.052
20.0	.05
21.0	.0476
22.0	.045

Distance from wire. Inches.	Turbidity. Reciprocal.
23.0	.043
24.0	.041
25.0	.04
26.0	.038
27.0	.037
28.0	.035
29.0	.034
30.0	.033
31.0	.0322
32.0	.031
33.0	.03
34.0	.029
35.0	.0285
36.0	.0277
37.0	.027
38.0	.0263
39.0	.0256
40.0	.025

GRADUATION OF U. S. GEOLOGICAL SURVEY ROD OF 1902.

Distance from wire. Millimeters	Turbidity.	Corresponding Reciprocal.
12.1	3 000	2.10
14.8	2 000	1.72
17.1	1 500	1.49
20.9	1 000	1.21
23.4	800	1.09
27.7	600	.92
30.9	500	.82

Distance from wire		Corresponding
Millimeters	Turbidity.	Reciprocal.
35.4	400	.72
38.8	350	.65
43.2	300	.59
49.1	250	.52
57.4	200	.44
62.4	180	.41
68.7	160	.37
72.0	150	.35
76.0	140	.334
81.0	130	.314
86.0	120	.295
90.0	110	.23
100.0	100	.254
105.0	95	.242
110.0	90	.230
116.0	85	.219
122.0	80	.208
130.0	75	.196
138.0	70	.184
147.0	65	.172
158.0	60	.16
171.0	55	.148
187.0	50	.136
205.0	45	.124
228.0	40	.111
257.0	35	.099
296.0	30	.086
314.0	28	.081
336.0	26	.076

361.0	24	.07
391.0	22	.065
426.0	20	.06
446.0	18	.057
468.0	18	.054

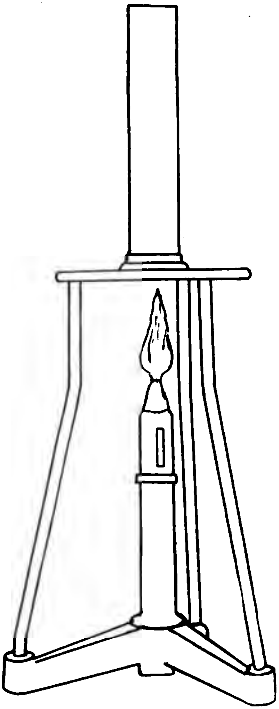


Fig. 12. **Jackson's Candle Turbidimeter.**

493.0	17	.052
520.0	16	.049
551.0	15	.046
587.0	14	.043
627.0	13	.041
674.0	12	.038
729.0	11	.035
794.0	10	.032
873.0	9	.029
971.0	8	.026
1 095.0	7	.023

METHOD WITH JACKSON'S TURBIDIMETER.

1 Place turbidimeter in dark room.
2 Have test water ready.
3 Light the candle, or turn on the electric bulb.
4 Pour in the water till the light just disappears.
5 Read the turbidity on the scale.

IN FIELD WORK.

1 Lower the electric turbidimeter into water.
2 Take the readings from the steel tape.

THE "SILICA STANDARD."

1 Prepare a fine silica from diatomaceous earth.
2 Must be so fine that,
    1. 1000 parts by weight per million,
    2 Make a turbidity of 1000.

3 Make up a series of jars of various turbidities.
4 Determine waters by comparison.

THE COEFFICIENT OF FINENESS.

1 Determine the total solids in liter of raw water.
2 Filter a liter of raw water.
3 Determine the total solids of filtered water.
4 The difference is suspended matter.
5 Determine the turbidity of raw water.
6 Then $\frac{\text{suspended matter}}{\text{turbidity}}$ equals the coefficient of fineness, or turbidity coefficient.

TOTAL SOLIDS.

1 100 c. c. water in weighed porcelain or platinum dish.
2 Evaporate to dryness.
3 Cool in desiccator.
4 Weigh and deduct weight of dish.
5 Multiply by 10 for contents of liter.

CHLORIN.

FIELD METHOD U. S. GEOL. SURVEY.

1 Take 100 c. c. water to be tested.
2 Add potassium chromate as an indicator.
3 Use silver nitrate tablets.
    1 Some equivalent to 1 mg. chlorin.
    2 Some equivalent to 10 mg. chlorin.

3 Prepared by Kremers-Urban Co. Mil. Wis.
4 Powder a tablet in glazed porcelain mortar.
5 Add to the water and stir.
6 Continue to add until red color appears, and is permanent.
7 Calculate the chlorin from number and value of tablets used.

TITRATION METHOD.

1 100 c. c. water in porcelain dish.
2 Add few drops potassium chromate as indicator.
3 Titrate with decinormal $AgNO_3$.
4 End point is a permanent red tinge.
5 Factor is .003545 for chlorin.

HARDNESS.

SODIUM OLEATE METHOD.

1 Use sodium oleate tablets of 10, 5 and 2.5 Mg.
2 100 c. c. water in bottle.
3 Add tablets one by one, dissolve and shake.
4 When foam appears, lay bottle on its side.
5 Foam should cover surface of water and persist five min.
6 Add smaller tablet, and repeat the process.
7 When standard foam is attained, estimate sodium oleate from tablets used.
8 Equivalents of sodium oleate tablets.
10 mg. tablet equals 1.38 mg. $CaCO_3$.
5 mg. tablet equals .68 mg. "
2.5 mg. tablet equals .31 mg. "

ALKALINITY.

SODIUM ACID-SULFATE METHOD.

1 100 c. c. water in porcelain mortar.
2 Add methyl orange as an indicator.
3 Add $NaHSO_4$ tablets of known strength.
4 Triturate tablets in mortar till dissolved.
5 Continue to end point, a faint pink.
6 Calculate to value of $CaCO_3$ marked on consignment of tablets.
7 Multiply by 10 for parts per million.

TITRATION METHOD.

1 100 c. c. water in beaker.
2 Two or three drops of methyl orange as indicator.
3 Titrate with decinormal H_2SO_4.
4 End point is a faint pink.
5 Multiply c. c. acid used by 50.
6 Result is $CaCO_3$ in parts per million.

CARBONATES AND BICARBONATES.

1 100 c. c. water in porcelain mortar.
2 Add 4 drops 1% phenolphthalein.
3 Add $NaHSO_4$ tablets and triturate in the mortar.
4 Continue till color disappears.
5 Calculate carbonates as follows:—
    1 Multiply number of tablets used by 2.
    2 Multiply by $CaCO_3$ value of each tablet.

    3 Multiply by factor 1.06.
    4 Multiply by 10.
    5 Result is parts per million in terms of sodium carbonate, Na_2CO_3.
6 Add few drops methyl orange to the water.
7 Add tablets and triturate as before.
8 End point is a faint pink.
9 Calculate bicarbonates as follows:—
    1 Take total number of tablets used.
    2 Take double the tablets used in first titration.
    3 Subtract.
    4 Multiply remainder by $CaCO_3$ value of tablet.
    5 Multiply result by 10.
    6 This gives parts per million.

TITRATION METHOD.

1 100 c. c. water in beaker.
2 Few drops phenolphthalein as indicator.
3 Titrate with decinormal $KHSO_4$ or $NaHSO_4$
4 Calculate carbonates as follows:—
    1 Take No. c. c. decinormal solution used.
    2 Multiply by factor .005.
    3 Multiply by 10; result is $CaCO_3$ in parts per million.
    4 Multiply by 1.06; result is $NaCO_3$.
5 Add few drops methyl orange as indicator.
6 Continue titration to faint pink.
7 Calculate bicarbonates as follows:—

1 Take No. c. c. for phenolphthalein end point.
2 Multiply by 2.
3 Take total c. c. used for both end points.
4 Subtract.
5 Multiply by factor .005 and by 10.
6 Result is parts per million $CaCO_3$.

SULFATES.

TURBIDITY METHOD.

1 Use 100 to 300 c. c. water.
2 Add one c. c. HCl for each 100 c. c. water.
3 Add one gram $BaCl_2$ crystals for each 100 c. c. water.
4 Shake frequently for 10 minutes.
5 Place in jar of Jackson turbidimeter, pouring in slowly till end point is reached.
6 Read turbidity in centimeters.
7 Estimate SO_3 by following table.

Reading in c.m.	Parts per Million SO_3	Reading in c.m.	Parts per Million SO_3	Reading in c.m.	Parts per Million SO_3
1.0	522	2.6	213	4.2	133
1.1	478	2.7	205	4.3	131
1.2	442	2.8	198	4.4	128
1.3	410	2.9	191	4.5	125
1.4	383	3.0	185	4.6	122
1.5	359	3.1	179	4.7	119
1.6	338	3.2	173	4.8	117
1.7	319	3.3	168	4.9	115
1.8	302	3.4	164	5.0	113
1.9	287	3.5	159	5.1	110

Reading in c.m.	Parts per Million SO_3	Reading in c.m.	Parts per Million SO_3	Reading in c.m.	Parts per Million SO_3
2.0	273	3.6	155	5.2	108
2.1	261	3.7	151	5.3	106
2.2	250	3.8	147	5.4	104
2.3	239	3.9	144	5.5	103
2.4	230	4.0	140	5.6	101
2.5	221	4.1	137	5.7	99
5.8	97	8.7	65	16.0	36
5.9	96	8.8	64	16.5	35
6.0	94	9.0	63	17.0	34
6.1	93	9.1	62	17.5	33
6.2	91	9.3	61	18.0	32
6.3	90	9.5	60	18.5	31
6.4	88	9.7	59	19.0	30
6.5	87	9.8	58	20.0	29
6.6	86	10.0	57	21.0	28
6.7	84	10.2	56	22.0	27
6.8	83	10.4	55	22.5	26
6.9	82	10.6	54	23.0	25
7.0	81	10.8	53	24.0	24
7.1	80	11.0	52	25.0	23
7.2	79	11.2	51	26.5	22
7.3	78	11.4	50	28.0	21
7.4	77	11.6	49	29.0	20
7.5	76	11.8	48	31.0	19
7.6	75	12.0	47	33.0	18
7.7	74	12.4	46	35.0	17
7.8	73	12.6	45	37.5	16
7.9	72	12.8	44	40.0	15
8.0	71	13.0	43	43.0	14
8.1	70	13.5	42	46.5	13

8.2	69	14.0	41	50.0	12
8.3	68	14.5	39	55.5	11
8.5	67	15.0	38	62.0	10
8.6	66	15.5	37	68.0	9

CALCIUM.

TURBIDITY METHOD.

1 Take 100 c. c. water.
2 Make alkaline with NH_4OH.
3 If precipitate forms, filter.
4 Add an excess of ammonium oxalate.
5 Mix thoroughly and let stand 15 minutes.
6 Take the turbidity with a Jackson turbidimeter.
7 Estimate the calcium in parts per million by the following table:

Reading in c.m.	Calcium.	Reading in c.m.	Calcium.	Reading in c.m.	Calcium.	Reading in c.m.	Calcium.
1.0	1 150	4.0	167	7.0	80	10.0	53
1.1	1 000	4.1	162	7.1	78	10.2	52
1.2	890	4.2	156	7.2	77	10.4	51
1.3	795	4.3	151	7.3	76	10.6	50
1.4	715	4.4	146	7.4	74	10.8	49
1.5	650	4.5	142	7.5	73	11.0	48
1.6	595	4.6	137	7.6	72	11.2	47
1.7	550	4.7	133	7.7	71	11.4	46
1.8	505	4.8	130	7.8	70	11.7	45
1.9	470	4.9	126	7.9	69	11.9	44
2.0	435	5.0	123	8.0	68	12.2	43
2.1	410	5.1	119	8.1	67	12.4	42
2.2	380	5.2	116	8.2	66	12.7	41
2.3	360	5.3	113	8.3	65	13.0	40

2.4	340	5.4	110	8.4	64	13.3	39
2.5	320	5.5	107	8.5	64	13.7	38
2.6	305	5.6	105	8.6	63	14.0	37
2.7	288	5.7	102	8.7	62	14.4	36
2.8	274	5.8	100	8.8	61	14.8	35
2.9	261	5.9	98	8.9	60	15.3	34
3.0	248	6.0	96	9.0	60	15.7	53
3.1	238	6.1	94	9.1	59	16.2	32
3.2	228	6.2	92	9.2	58	16.7	31
3.3	218	6.3	90	9.3	57	17.3	30
3.4	209	6.4	88	9.4	57	17.9	29
3.5	200	6.5	87	9.5	56	18.5	28
3.6	194	6.6	85	9.6	55	19.2	27
3.7	186	6.7	84	9.7	55	20.0	26
3.8	179	6.8	82	9.8	54	21.7	24
3.9	173	6.9	81	9.9	54	22.7	23

COLOR.

1 1.246 gram potassium-platinic chlorid ($PtCl_4$, 2KCl).
2 1 gram crystallized cobalt chlorid ($CoCl_2$, $6H_2O$).
3 Dissolve in water with 100 c. c. HCl.
4 Make up to liter with distilled water.
5 This makes a standard of 500 in color.
6 Dilute and make other standards as 5, 10, 15, 20, 30, 40, 50, 60, and 70.
7 Keep the standards in 100 c. c. Nessler jars with about 20 to 25 c. m. depth of liquid.
8 Fill similar jars with water to be tested and compare.

9 Filter turbid water through filter paper before testing.

IRON.

1 Dissolve .1 gram piano wire in HNO_3.
2 Make up to a liter with distilled water.
3 Add 20 grams KSCN.
4 This standard iron sol. will have .1 mg. iron to c. c.

KSCN SOLUTION.

1 Take 20 grams KSCN.
2 Dissolve in liter of water.

PROCESS.

1 45 c. c. water in a Nessler tube.
2 Add 2 c. c. nitric acid.
3 Mix and let stand 5 minutes.
4 Add 3 c. c. KCSN sol.
5 Mix and let stand 10 minutes.
6 Take another Nessler tube.
7 Fill to 45 c. c. with distilled water.
8 Run in standard iron solution from burette.
9 When colors exactly match, read amount sol. used from burette.
10 Calculate the percentage and parts per million.

MINERAL ANALYSIS OF WATER.

1 1000 c. c. water in a beaker.
2 Evaporate to dryness.
3 Moisten the residue with HCl.
4 Add 25 to 50 c. c. distilled water and boil.

5 Filter, wash with hot distilled water.
6 Burn in porcelain cruicible and weigh as silica (SiO_2).
7 Add NH_4OH to filtrate.
8 Heat to boiling.
9 Permit precipitate to settle.
10 Filter and wash filter.
11 Burn in porcelain crucible.
12 Cool and weigh as alumina and ferric oxid (Al_2O_3 and Fe_2O_3).
13 Evaporate filtrate to about 100 c. c.
14 Make alkaline with NH_4OH.
15 Add excess of ammonium oxalate.
16 Boil 15 to 30 min.
17 Permit to settle.
18 Filter.
19 Burn and blast to constant weight.
20 Cool and weigh as CaO.
    1 Evaporate filtrate to small amount.
    2 Add ammonia and sodium phosphate.
    3 Stir and set in cool place 6 hours.
    4 Filter and wash with weak ammonia.
    5 Burn and blast to constant weight.
    6 Cool and weigh as magnesium pyrophosphate.
    7 Factor for MgO is .36036.

SULFURIC ACID.

1 One to five liters of water.
2 Acidulate with HCl.
3 Evaporate to dryness.
4 Moisten with HCl and dissolve in distilled water.

5 Filter off the silica.
6 Wash filter with hot distilled water.
7 Heat to boiling and add excess of $BaCl_2$.
8 Boil and set aside to settle.
9 Filter and wash filter.
10 Burn in porcelain crucible.
11 Add 2 or 3 drops H_2SO_4.
12 Heat till sulfur fumes cease.
13 Cool and weigh as $BaSO_4$.
14 Factor is .3433 for SO_3.

POTASSIUM AND SODIUM.

1 One to 5 liters of water.
2 Acidulate with HCl.
3 Evaporate to 75 c. c.
4 Add barium hydrate as long as a precipitate forms.
5 Heat to boiling, filter and wash filter.
6 Evaporate filtrate to 75 c. c.
7 Add few drops ammonia.
8 Add strong ammonium carbonate as long as a precipitate forms.
9 Let solution stand for some time, stir occasionally.
10 Filter and wash filter with weak ammonia and ammonium carbonate solution.
11 Add few drops HCl and evaporate to dryness.
12 Heat to 100 C. and gradually heat to 130 C.
13 Then cautiously heat to dull redness.
14 Dissolve in 10 c. c. distilled water, warm gently.

15 Add few drops barium hydrate and ammonium carbonate.
16 Stand short time and stir.
17 Filter and wash as before.
18 Add few drops HCl and evaporate to dryness in weighed porcelain crucible.
19 Heat slowly to 100 C. then to 130 C.
20 Heat to a dull red to expel ammonium salts.
21 Cool in desiccator and weigh at once.
22 The weight is the combined sodium and potassium chlorides.

THE SEPARATION.

1 Dissolve in least possible amount of distilled water.
2 Treat with sol. platinum chloride.
3 Evaporate to pasty consistency with little boiling.
4 Cool and add 35 c. c. 80% alcohol.
5 Let stand in warm place 1 hour, stirring occasionally.
6 Dry and weigh a filter paper.
7 Filter and wash with 80% alcohol.
8 Dry at 120 C.
9 Weigh as K_2PtCl_6.
10 Factor is .3 for KCl.
11 Subtract KCl from the combined salts.
12 The remainder is NaCl.

COAGULATION AND PRECIPITATION.

1 Estimate the alkalinity of the water.
2 Add a chemical equivalent of calcium oxid or calcium hydrate.

3 Add a chemical equivalent of aluminum sulfate.
4 Mix and let stand to precipitate.

FILTRATION.

1 Make the above preparation over a sand bed in a filter.
2 Permit to settle.
3 Open the filter and run first water to waste.
4 Continue the filtration.
5 The filtrate will be perfectly clear and clean.
6 Additional water will be well filtered.

SOFTENING.

1 Calculate the total hardness of the water.
2 Add a chemical equivalent of barium hydrate.
3 Permit to settle.
4 Decant or draw off through a decanting filter.
1 Calculate the total hardness of the water.
2 Add a chemical equivalent of barium hydrate.
3 Add a small portion of lime and aluminum sulfate.
4 Permit to settle on sand bed of filter.
5 Filter through sand.

BACTERIA AND B.COLI.

DETECTION OF SEWAGE.

Use the methods for B.Coli and B.Typhosus and the methods for counting bacteria given under Bacteriology, Chapter VI.

EXAMINATION OF MILK.

COW'S MILK.

SPECIFIC GRAVITY.

1 Use cylinder holding 500 c. c.
2 Fill two-thirds full of milk.
3 Use lactometer.
4 Temperature should be 60 F.

NORMAL SPECIFIC GRAVITIES.

1 Whole milk. . . . . .1.028 to 1.033.
2 Skimmed milk. . . . . .1.035 to 1.0365.

TOTAL SOLIDS.

1 Use a weighed aluminum dish.
2 Add 50 or 100 grams milk.
3 Evaporate to dryness without scorching.
4 Weigh.
5 Deduct weight of dish.

ASH.

1 50 grams milk in weighed porcelain crucible.
2 Evaporate to dryness.
3 Place in muffle furnace and burn to ash.
4 Cool and weigh.
5 Deduct weight of crucible.

ACIDITY.

1 Take 10 c. c. milk.
2 Add 90 c. c. distilled water.
3 Add two or three drops phenolphthalein.
4 Titrate with decinormal NaOH.

FATS.

FESER'S METHOD.

1 Use Feser's Lactoscope.
2 Place 4 c. c. milk in instrument.
3 Add water slowly and mix well.

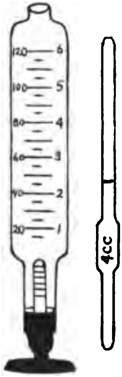


Fig. 13. Feser's Lactoscope.

4 Continue process till black lines of the pillar are distinct.
5 Read percentage of fat at top of liquid.

THE BABCOCK TEST.

1 Use a Babcock machine and tubes.
2 Place 17.6 c. c. milk in graduated Babcock tube.
3 Add 17.5 c. c. sulfuric acid.
4 Shake and mix well.
5 Place in Babcock machine.

6 Centrifugate 5 minutes.
7 Add boiling water to bring fats within graduations.
8 Centrifugate one minute.
9 Read percentage of fats.
10 For fat in cream, use the tube for cream.

ETHER EXTRACTION PROCESS.

1 Weigh out 5 grams of milk.
2 Place in Hofmeister schalchen on ignited asbestos.
3 Dry.
4 Crush the schalchen in a Schleicher & Schull shell.
5 Place shell in Soxhlet tube.
6 Extract in Soxhlet apparatus.
7 Evaporate remaining ether to dryness.
8 Weigh flask and residue.
9 Deduct weight of flask.
10 Calculate the percentage of fat.

WERNER-SCHMIDT METHOD.

1 10 c. c. milk in 50 c. c. tt.
2 Add 10 c. c. concentrated HCl.
3 Shake and heat till brown.
4 Immerse in cold water to cool.
5 Add 30 c. c. ether.
6 Shake.
7 Permit ether to separate.
8 Withdraw ether into weighed flask.
9 Repeat twice with 10 c. c. ether.
10 Distil off the ether.

11 Weigh the flask and contents.
12 Deduct weight of flask.

THE ADAMS METHOD.

1 Strip of filter paper 2.5 by 22 inches.
2 Roll into coil and secure with wire.
3 About 5 c. c. milk in beaker. Weigh.
4 Place the filter coil in the beaker.
5 Hold it by the wire till milk is absorbed.
6 Withdraw coil and hang in air to dry.
7 Weigh the beaker.
8 Estimate milk absorbed by coil.
9 When coil is dry in air,
10 Continue drying in oven below 100 C.
11 Place coil in Soxhlet apparatus.
12 Etract with ether at least 2 hours.
13 Evaporate the ether from the flask.
14 Weigh the flask and contents.
15 Deduct weight of flask.

SOLIDS NOT FAT.

Subtract the fat from the total solids.

PROTEIDS.

WOODWARD'S METHOD.

1 Use two Woodward's milk burettes.

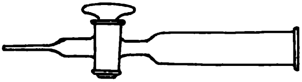


Fig. 14. Woodward's Milk Burette.

2 Place 5 c. c. milk in each.

3 Keep at 37 to 40 C. 18 to 24 hours.
4 Cool the tubes.
5 Withdraw the fluid into a graduated Purdy tube.
6 Add Esbach's solution to 15 c. c. mark.
7 Mix and let stand.
8 Centrifugate to constant reading.

RITTHAUSEN'S METHOD.

1 10 grams of milk in a beaker.
2 Add 90 c. c. water.
3 Add 5c. c. Fehling's copper sol. and stir.
4 Add slowly 2.5% NaOH sol. till sol. is nearly neutral.
5 Allow to settle.
6 Dry and weigh a filter.
7 Decant through this filter.
8 Wash by decantation.
9 Transfer precipitate to filter.
10 Wash with water, drain dry.
11 Wash with strong alcohol and dry.
12 Extract with ether.
13 Dry filter in oven at 130 C.
14 Weigh.
15 Deduct weight of filter.
16 Burn to ash in porcelain crucible.
17 Deduct weight of the ash.

CASEIN.

1 10 grams of milk in a beaker.
2 Add 90 c. c. water at 40 to 42 C.
3 Add 1.5 c. c. 10% sol. acetic acid.

4 Stir, keep warm and let stand till precipitate settles.
5 Decant on filter, wash with cold water.
6 Transfer precipitate to filter and wash.
7 Transfer filter and precipitate to Kjeldahl flask.
8 Obtain the nitrogen by Kjeldahl process.
9 N x 6.38 equals casein.

SUGAR.

MILK SUGAR SOLUTION.

1 25 grams or 24.2 c. c. milk in 250 c. c. flask.
2 Add .5 c. c. 30% acetic acid and shake.
3 Let stand few minutes.
4 Run in 100 c. c. boiling water and shake.
5 Add 25 c. c. alumina cream and shake again.
6 Set aside ten minutes.
Prepare a wet ribbed filter.
Decant upon filter and finally filter contents of flask.
Filtrate and washing should equal 250 c. c.
Filtrate must be perfectly clear.

DETERMINATION.

5 c. c. Fehling No. 1 and 5 c. c. Fehling No. 2 in porcelain dish.
Bring to a boil.
Run in milk sugar solution from burette.
Continue till blue color disappears.
Read amount sugar solution from burette.
Repeat the process several times and take average.

7 Then $\dfrac{250}{\text{c. c. used}} \times .067$ equals grams milk sugar in 25 grams milk.
8 Multiply by 4 for the percentage.

ADDED WATER.

THE MILK SERUM.

1 100 c. c. milk at 20 C. in a beaker.
2 Add 2 c. c. 25% acetic acid.
3 Cover with a watch glass.
4 Heat in water bath 20 min. at 70 C.
5 Place in ice water 10 min.
7 Filter.

IMMERSION REFRACTOMETER METHOD.

1 Place beaker in refractometer bath.
2 Bring temperature to 20 C.
3 Immerse glass of refractometer in milk serum.
4 Take the reading.
5 Compare with table for added water.

SKIMMED MILK.

Refractometer reading.	Added water.
42.85	0%
39.60	10
36.85	20
34.00	30

31.20	40
28.50	50

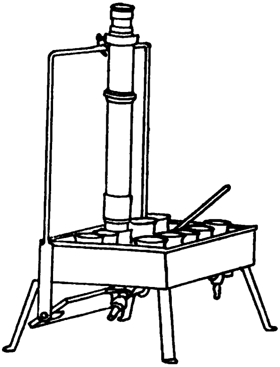


Fig. 15. Zeiss Immersion Refractometer.

WHOLE MILK.

Refractometer reading.	Added water.
42.40	0%
39.75	10
36.90	20
34.10	30
31.10	40
28.45	50

FORMALDEHYDE IN MILK.

HCL TEST.

1 Use commercial HCl containing 2 c. c. 10% ferric chlorid per liter.

2 10 c. c. milk in porcelain casserole.
3 10 c. c. of the HCl.
4 Heat nearly to boiling.
5 Hold casserole by the handle and give rotary motion.
6 Violet color indicates formaldehyde.
7 Will detect one part in 250,000 in sweet milk.
8 Will detect one part in 50,000 in sour milk.

H_2SO_4 TEST.

1 10 c. c. milk in wide test tube.
2 Underlay with 5 c. c. sulfuric acid by carefully running down side of tube.
3 A violet line of contact indicates formaldehyde.
4 Not so sensitive as HCl test.
5 Charring action of sulfuric acid masks the reaction.

BENZOIC ACID.

1 50 c. c. milk in a flask.
2 Add 5 c. c. HCl. Shake.
3 Add 150 c. c. ether, cork flask and shake.
4 Centrifugate or shake with large amount of ether to break up emulsion.
5 Transfer ether extract to separatory funnel.
6 Add dilute ammonia and shake.
7 Withdraw the ammonia solution.

8 Evaporate ammonia solution over water bath till ammonia disappears.
9 Add few drops ferric chlorid.
10 Benzoic acid gives characteristic flesh colored precipitate.

SALICYLIC ACID.

1 Proceed exactly as for benzoic acid.
2 Ferric chlorid gives a violet color in presence of salicylic acid.

BACTERIA IN MILK.

1 Make a dilution of 1 : 1000 in sterile distilled water.
2 Make the following determinations as directed in chapter VI.

    1 Petri plate cultures.
    2 Count the bacteria.
    3 Isolate the bacillus coli.

B. TUBERCULOSIS IN MILK.

1 Fill centrifuge tubes with various dilutions of milk.
2 Centrifugate.
3 Make smears of the sediment.
4. Stain by the Ziehl-Neelson method.

1 Feed the suspected milk to Guinea pigs.

2 Inoculate Guinea pigs with the sediment from the centrifuge.
3 Make pathological examination of the dead pig.

NUMBER OF BACTERIA IN MILK.

MILK COLLECTED WITH GREAT CARE.

Kept at:—

	4 C	6 C	13 C	20 C
24 hours.	2500	3100	18800	450000
48 "	3600	12000	3400000	
96 "	218000	1480000		
168 "	4209000	80000000		

ORDINARY MILK.

	4 C	6 C	13 C	20 C
24 hours.	38000	42000	187000	4000000
48 "	56000	360000	38000000	
96 "	4300000	12200000		
168 "	38000000	300000000		

BACTERIAL STANDARDS FOR MILK.

New York Milk Commission......	30 000 c. c.
Philadelphia Milk Commission.....	10 000 c. c.
Boston.	500 000 c. c.
Milwaukee.	250 000 c. c.
Rochester, N. Y.	100 000 c. c.

SANITARY EXAMINATION OF DAIRIES, MILK DEPOTS AND SALESROOMS.

DAIRY SCORES.

DETAILED SCORE

EQUIPMENT		SCORE	
		Perfect	All'w'd
COWS.			
Condition		4	
Health (outward appearance)		6	
Comfort		4	
Bedding	2		
Temperature of stable	1		
Protected yard	1		
Cubic feet of space per cow: Over 300, 2; over 400, 4; 500 to to 1,000, 6		6	
Feed		4	
Water		8	
Clean	6		
Fresh	2		
STABLE			
Location		6	
Well drained	3		
Free from contaminating surroundings	3		
Construction		10	
Tight, sound floor	3		
Gutter	1		
Stall, stanchion, tie	1		
Low down manger	1		
Smooth, tight walls	1		
Smooth, tight ceiling	2		
Box stall	1		
Light; 1 sq. ft. glass per cow, 2; 2 sq. ft., 4; 3 sq. ft., 6; 4 sq. ft., 8; even distribution, 2		10	
Ventilation; Sliding windows, 2; hinged at bottom, 4; King system or muslin curtain, 8		8	
Stable yard (drainage)		2	
MILK ROOM.			
Location		6	
Convenience	2		
Free from contaminating surroundings	4		
Construction		4	
Floor	1.5		
Walls and ceilings	1		
Light	.5		
Ventilation	.5		
Screens	.5		
Arrangement		2	
Equipment		6	
Hot water or steam	2		
Cooler	2		
Narrow top milk pail	1		
Other utensils	1		
Water supply for utensils		10	
Clean	6		
Convenient	2		
Abundant	2		
Milking suits		4	
Total		100	
DEDUCTIONS.			
Cows not tuberculin tested		25	
Diseased udders		25	
Filthy water		20	
Other deductions			
Total deductions			
Net total			

METHODS		SCORE	
		Perfect	All'w'd
Cleanliness of cows		10	
Cleanliness of stable		12	
Floor	4		
Walls	3		
Ceiling	2		
Ledges	1		
Mangers and partitions	1		
Windows	1		
Stable air		4	
Removal of manure		4	
To field or proper pit	4		
30 feet from stable	2		
Cleanliness of stable yard		2	
Cleanliness of milk room		6	
Care and cleanliness of utensils		10	
Inverted, in pure air	2		
Clean (sterilized)	8		
Cleanliness in milking		14	
Clean, dry hands	4		
Udders washed and dried	10		
Cleaned with moist cloth	8		
Cleaned with dry cloth	4		
Cooling		20	
Cooled immediately after milking each cow	5		
Cooled to 50° F. or below	8		
51° to 55° F	6		
56° to 60° F	4		
Storing		8	
Below 50° F	8		
51° to 55° F	6		
56° to 60° F	4		
Transportation		10	
Iced	10		
Jacket or wet blanket	8		
Dry blanket	4		
Covered wagon	2		
Total		100	
DEDUCTIONS.			
Any attendant with contagious disease, not isolated		25	
Filthy udders during milking		20	
Milking with wet hands		10	
Other deductions			
Total deductions			
Net total			

DESCRIPTION	SCORE		REMARKS
	PERFECT	ALLOWED	
STORE LOCATION---Deduct when not separated by partition from Living Rooms 3, Kitchen 3, Laundry 5, Barn 5.	5		
LIGHT and VENTILATION---If window space is equivalent to 15 per cent of floor space, allow Deduct 1 point for every 3 per cent. less than above amount.	5		
CLEANLINESS---Deduct for Unclean Floor 3, Unclean Walls, 3, Unclean Shelves 3, Unnecessary Articles and Rubbish 3.	10		
MILK BOX LOCATION---Deduct when near Door of Living Rooms 2, Kerosene Barrel 3, Rubbish, etc., 3.	5		
CONSTRUCTION --- Deduct when Lining defective 3, Drainage defective 3, Cover defective 2.	5		
CLEANLINESS---Make deductions according to amount.	10		
ODOR---If none allow-	5		
CONTENTS, other than Milk---Deduct, Meat 2, Cheese 3, Miscellaneous 5.	5		
UTENSILS CONDITION--Deduction when rusty, worn out or damaged.	5		
CONSTRUCTION --- Deduct when poor from sanitary standpoint	10		
CLEANLINESS---Deduct according to amount.	10		
MILK HANDLING--If cooled promptly to 50° F. allow 10 points. If Pasteurized and promptly cooled allow 10 points. If improperly bottled or otherwise poorly handled deduct accordingly	10		
STORAGE---If stored at 50° F. or below allow 10. Deduct 1 point for every 3 degrees above 50° F.	10		
ATTENDANT Cleanliness	5		
TOTAL	100		

SCORES OF MILK DEPOT.

DESCRIPTION	SCORE		REMARKS
	PERFECT	ALLOWED	
MILK and SALESROOM LOCATION --- Deduct when connected with Store 2, Living Rooms 8, Kitchen 5, Laundry 8, Barn 8.	10		
CONSTRUCTION --- Floor, if cement, etc., 3, Walls and Ceilings 3, Drainage 4.	10		
CLEANLINESS---Deduct when Unclean Floor 5, Unclean Walls 4, Unnecessary Articles 3, Odor 3.	15		
LIGHT and VENTILATION--- If window space is equivalent to 15 per cent or more of the floor space, allow 10. Deduct 2 points for every 3 per cent less than the above amount.	10		
EQUIPMENT --- Arrangement 3, Sanitary Construction 4, Condition 3, Cleanliness 5.	15		
WASHROOM Location 2, Construction 2, Equipment 3, Cleanliness 3, (If none allow 0.)	10		
MILK HANDLING--If cooled promptly to 50° F, allow 10 points. If Pasteurized and promptly cooled allow 10 points. If improperly bottled or otherwise poorly handled deduct accordingly.	10		
STORAGE---If stored at 50° F or below allow 10. Deduct 1 point for every 3 degrees above 50° F.	10		
Wagons and Delivery General Appearance 2, Protection of Product 3, Cleanliness 5.	10		
TOTAL	100		

SCORES OF STORES SELLING MILK.

HUMAN MILK.

1 Amount. 500 to 1500 c. c. in 24 hours.
2 Reaction. Alkaline.
3 Specific gravity. 1.026 to 1.035.
1.028 to 1.034.

ANALYSIS OF HUMAN MILK.

Water	876.00	891.00	872.40	892.00	890.60
Solids	124.00	109.00	127.00	108.00	109.40
Albumin	22.10	17.90	19.00	16.13	17.24
Fat	38.10	33.00	43.20	32.28	29.15
Lactose	60.90	53.90	59.80	57.94	59.92
Salts	2.90	4.20	2.60	1.65	2.09

Fig. 16. Tube for Human Milk.

COW'S MILK.

Water	874.2	
Solids	125.8	
Casein	28.8	
Albumin	5.3	34.1
Fat	36.6	
Lactose	48.1	
Salts	7.1	

SPECIFIC GRAVITY.

1 Bring the milk to 60 F. in water bath.
2 Use Quevenne's lactodensimeter.

1 Use the Feser method as for cows' milk.

FAT.

BABCOCK METHOD.

1 Use small tube for human milk.
2 Use 5 c. c. milk.
3 Add sulfuric acid to 0 mark.
4 Mix by shaking and rotating.
5 Centrifugate in urinary centrifuge.

LEFFMANN-BEAM METHOD.

1 Use the small tube for human milk.
2 Use exactly 5 c. c. milk.
3 Add 1 c. c. of an equal mixture of HCl and amyl alcohol.
4 Shake and mix.
5 Add sulfuric acid slowly to 0 mark.
6 Shake and mix.
7 Centrifugage 3 min. at 1000 R.P.M.
8 Read percentage of fat in graduations.

PROTEIDS.

Apply Woodward's Method.

T. R. BOGGS' MODIFICATION.

REAGENTS.

1	Phosfotungstic acid	25 grams.
2	Distilled water	125 c. c.
1	Concentrated HCl	25 c. c.
2	Distilled water	100 c. c.

1 After preparation, mix the two solutions.
2 Keep in dark bottle in dark place.

PROCESS.

1 Dilute milk.
    1 Human milk 1 : 10.
    2 Cows' milk 1 : 20.
2 10 c. c. diluted milk in graduated Purdy tube.
3 Add 5 c. c. Boggs' solution.
4 Mix and let stand to settle.
5 Centrifugate to constant volume.
6 Read percentage of proteids.
7 About 80% of proteids is casein.
8 About 15% is albumin.

CHAPTER V.

URINALYSIS.

THE PATIENT.

1 Age.
2 Sex.
3 Weight.
4 Occupation.
5 Married or single.
6 Habit.
7 Nourishment.
8 Symptoms.
9 Physical diagnosis.

THE SAMPLE.

1 Fresh.
2 Passed in 24 hours.
3 Amount in 24 hours.

THE EXAMINATION.

PHYSICAL.

1 Color—by Vogel's Scale.
2 Odor.
3 Transparency.
4 Opacity, due to
  1 Amorphous phosphates.
  2 Amorphous urates.

3 Pus.
4 Mucus.
5 Blood.
6 Bacteria.

SPECIFIC GRAVITY.

1 Use a good urinometer.
2 Fill cylinder about two-thirds full of urine.
3 Introduce urinometer to near the bottom of the urine.
4 Urinometer will rise and seek its level.
5 Read the figures at surface of urine.

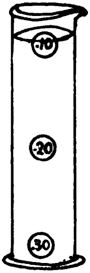


Fig. 17. Specific Gravity **Method With Beads.**

WITH SPECIFIC GRAVITY BEADS.

1 Place urine in a cylinder.
2 Introduce a number of the beads.
3 Take the number on the bead suspended midway.

SPECIFIC GRAVITY OF URINE.

1 Normal. . . . . .1.015 to 1.025.
2 Pathologic. . . . . .1.001 to 1.06.

THE CHEMICAL EXAMINATION.

THE REACTION.

1 Test first with blue litmus paper.
2 Samples negative to blue litmus must be tested with red litmus.

TOTAL SOLIDS.

1 Normal amount, 60 grams to 150 pounds weight.
2 One-half this amount after 75 years.

APPROXIMATE ESTIMATION.

1 Take the specific gravity..
2 Take the last two figures of the specific gravity as a whole number.
3 Multiply by 2.33.
4 Multiply by the whole number of c. c. passed in 24 hours.
5 Divide by 1000. Result is total solids in grams.
6 Example.
Specific gravity is 1.020.
Total amount is 1500 c. c.
20 x 2.33 are 46.60.
$46.6 \text{ x } \frac{1500}{1000}$ are 69.90 grams in 24 hours.

PARKE'S TABLE.

Water	1500.00	c. c.
Total solids	72.00	grams.
Urea	33.18	"
Uric acid	.55	"
Hippuric acid	.40	"

Kreatinin	.91	“
Pigments and other organic matter	10.00	“
Sulfuric acid	2.01	“
Phosphoric acid	3.16	“
Chlorin	7-8.00	“
Ammonia	.77	“
Potassium	2.50	“
Sodium	11.09	“
Calcium	.26	“
Magnesium	.21	“

GRAVIMETRIC ESTIMATION.

1 100 c. c. fresh urine in weighed porcelain dish.

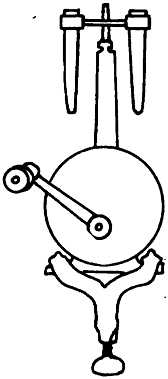


Fig. 18. Hand Centrifuge.

2 Evaporate to dryness on water bath.

3 Continue drying carefully in oven at 100 C. to constant weight.
4 Cool in desiccator.
5 Weigh. Calculate to total amount.

MINERAL ASH.

1 100 c. c. fresh urine in weighed porcelain dish.
2 Evaporate to dryness at 100 C.
3 Dissolve residue in boiling distilled water.
4 Filter and wash filter with hot water.
5 Burn the filter to white ash.
6 Place ash, filtrate and washings in dish again.
7 Evaporate to dryness at 100 C.
8 Cool in desiccator and weigh.

MINERAL CONSTITUENTS.

CHLORIDS.

PURDY'S METHOD.

1 10 c. c. filtered urine in graduated Purdy tube.
2 1 c. c. HNO_3.
3 4 c. c. $AgNO_3$.
4 Mix.

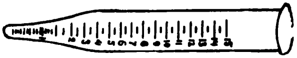


Fig. 19. Graduated Purdy Tube.

5 Let stand three minutes.
6 Centrifugate 3 minutes at 1200 R.P.M.
7 Read bulk percentage.

8 Estimate by following table.

Bulk %	NaCl %	Grams NaCl per liter.
.25	.03	.3
.50	.07	.7
.75	.1	1.0
1.00	.13	1.3
1.25	.16	1.6
1.50	.19	1.9
1.75	.23	2.3
2.00	.26	2.6
2.25	.29	2.9
2.50	.32	3.2
2.75	.36	3.6
3.00	.39	3.9
3.25	.42	4.2
3.50	.45	4.5
3.75	.49	4.9
4.00	.52	5.2
4.25	.55	5.5
4.50	.58	5.8
4.75	.62	6.2
5.00	.65	6.5
5.50	.71	7.1
6.00	.78	7.8
6.50	.84	8.4
7.00	.91	9.1
7.50	.97	9.7
8.00	1.04	10.4
8.50	1.1	11.0
9.00	1.17	11.7
9.50	1.23	12.3
10.00	1.3	13.0

Bulk %	NaCl %	Grams NaCl per liter.
10.50	1.36	13.6
11.00	1.43	14.3
11.50	1.49	14.9
12.00	1.56	15.6
12.50	1.62	16.2
13.00	1.69	16.9
13.50	1.75	17.5
14.00	1.82	18.2
14.50	1.88	18.8
15.00	1.94	19.4
15.50	2.01	20.1
16.00	2.07	20.7
16.50	2.14	21.4
17.00	2.20	22.0
17.50	2.27	22.7
18.00	2.33	23.3
18.50	2.40	24.0
19.00	2.46	24.6
19.50	2.53	25.3
20.00	2.59	25.9

GRAVIMETRIC METHODS.

1 Take 10 c. c. filtered urine.
2 Dilute to 100 c. c. with distilled water.
3 Make distinctly acid with HNO_3.
4 Precipitate with $AgNO_3$.
5 Filter and wash the filter.
6 Scrape precipitate into weighed porcelain crucible.
7 Burn filter paper to ash and add to crucible.
8 Dry precipitate and heat to incipient fusion.

9 Weight as silver chloride (AgCl).
10 Multiply by .408. The result will be the weight of sodium chloride in 10 c. c. of urine.
11 Multiply by 100 for the amount per liter.

1 Transfer the silver chloride of the last determination to a bone ash cupel.
2 Place in muffle furnace and blast to pure silver.
3 Permit silver button and cupel to cool in furnace.
4 Weigh the silver button.
5 Multiply by .542. The result is the weight of sodium chloride in 10 c. c. of urine.

CHLORIDES—TITRATION METHOD.

1 10 c. c. filtered urine.
2 Make up to 100 c. c. with distilled water.

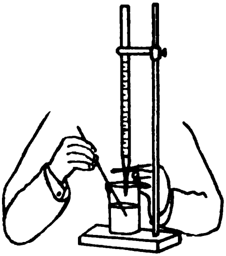


Fig. 20. Titration.

3 Add few drops sol. potassium chromate as indicator.

4 Titrate with N/10 $AgNO_3$ to a light red.
5 Use factors .00585 for NaCl and .003545 for Cl.
The results thus obtained are too high.

NEUBAUER-SALKOWSKI METHOD.

1 10 c. c. filtered urine.
2 Add 1 gram Na_2CO_3 and 3 to 5 grams KNO_3.
3 Evaporate to dryness in a crucible below 100 C.
4 Ash at dull red heat.
5 Dissolve in distilled water.
6 Neutralize with very dilute HNO_3.
7 Add few drops potassium chromate as indicator.
8 Titrate with N/10 $AgNO_3$ till faint red color is permanent on stirring.
9 Use factors given above.

SALKOWSKI-VOLHARD METHOD.

REAGENTS.

1 N/10 $AgNO_3$.
2 N/10 KSCN.
3 Sat. Sol. ammonio-ferric alum.
4 HNO_3 sp. gr. 1.2.

PROCESS.

1 10 c. c. filtered urine in stoppered flask with 100 c. c. mark.
2 Add 50 c. c. distilled water and 4 c. c. HNO_3.

3 Run in N/10 $AgNO_3$ in excess from burette, noting amount.
4 Mix well and add water to 100 c. c.
5 Filter through dry filter into dry beaker.
6 Add 5 c. c. ferric alum sol.
7 Titrate with KSCN sol. to end point, a slight red tinge.
8 Subtract No. c. c. KSCN used from No. c. c. $AgNO_3$ used.
9 Use same factors as above.

SULFATES.

PURDY'S CENTRIFUGAL METHOD.

1 10 c. c. filtered urine in graduated Purdy tube.
2 Add 5 c. c. barium chlorid mixture.

1	Sat. Sol. $BaCl_2$	40 c. c.
2	HCl	10 c. c.
3	Distilled water	160 c. c.

3 Mix and let stand 3 to 5 minutes.
4 Centrifugate 3 min. at 1200 R.P.M.
5 Read percentage of sulfates.
6 Estimate by following table.

PURDY'S TABLE OF SULFATES.

Bulk % $BaSO_4$.	Per cent SO_3.	Grams SO_3 per liter.
.125	.04	.4
.25	.07	.7
.375	.1	1.0
.5	.13	1.3

.625	.16	1.6
.75	.19	1.9
.875	.22	2.2
1.0	.25	2.5
1.25	.31	3.1
1.5	.37	3.7
1.75	.43	4.3
2.0	.49	4.9
2.25	.55	5.5
2.5	.61	6.1
2.75	.67	6.7
3.0	.73	7.3
3.25	.79	7.9
3.5	.85	8.5
3.75	.91	9.1
4.0	.97	9.7
4.25	1.03	10.3
4.5	1.09	10.9
4.75	1.15	11.5
5.0	1.21	12.1

TOTAL SULFATES.

GRAVIMETRIC METHOD.

1 100 c. c. filtered urine in beaker.
2 Add 8 c. c. HCl and boil over water bath.
3 Add 20 c. c. saturated solution $BaCl_2$.
4 Continue boiling about 30 minutes.
5 Filter.
6 Wash filter with boiling water.
7 Wash with hot 70% alcohol.
8 Wash with ether.
9 Remove precipitate to porcelain crucible

10 Ash filter separately and add ash to precipitate.
11 Burn to constant weight.
12 Cool and weigh as $BaSO_4$.
13 Multiply by .3433 for SO_3, or by .1373 for S.

ETHEREAL SULFATES.

1 100 c. c. filtered urine in beaker.
2 Add 100 c. c. alkaline solution $BaCl_2$.
3 Stir thoroughly.
4 Filter into graduate to 100 c. c. mark.
5 Make strongly acid with HCl.
6 Boil over water bath.
7 Filter, wash, dry and weigh as above.
8 Multiply by 2, then use factors as above.

MINERAL SULFATES.

Subtract the Ethereal Sulfates from the Total Sulfates.

TOTAL SULFUR.

REAGENTS.

1 Mix Na_2CO_3 and K_2CO_3 in ratio of 11 to 14.
2 Crystalline potassium permanganate.
3 Concentrated HCl.
4 Saturated solution $BaCl_2$.

PROCESS.

1 100 c. c. filtered urine.
2 Add 12 grams of the carbonate mixture.
3 Evaporate to dryness in nickel crucible.

4 Fuse, cool and extract with hot water.
5 Filter. Wash.
6 Treat filtrate with few crystals of permanganate.
7 Heat 15 minutes and repeat last step.
8 Render distinctly acid with HCl.
9 Boil and treat with 20 c. c. $BaCl_2$.
10 Proceed as above.

PHOSFATES.

PURDY'S CENTRIFUGAL METHOD.

TOTAL PHOSFATES.

1 10 c. c. filtered urine in graduated Purdy tube.
2 2 c. c. 50% acetic acid.
3 3 c. c. 5% solution uranium nitrate.
4 Mix and let stand three minutes.
5 Centrifugate three minutes at 1200 R.P.M.
6 Read bulk percentage.

PHOSFATES OF CALCIUM AND MAGNESIUM.

(EARTHLY PHOSFATES.)

1 10 c. c. filtered urine in graduated Purdy tube.
2 5 c. c. NH_4OH.
3 Mix and warm.
4 Let stand three minutes.
5 Centrifugate three minutes at 1200 R.P.M.
6 Read bulk percentage.

PHOSFATES OF SODIUM AND POTASSIUM.

(ALKALINE PHOSFATES.)

Take the difference between the above determinations.

Percentage of phosforic acid is about 1/85 of the bulk percentage. Estimate by

PURDY'S TABLE OF PHOSFATES.

Bulk %	P_2O_5 %	Grams P_2O_5 per liter.
.5	.02	.2
1.0	.04	.4
1.5	.045	.45
2.0	.05	.5
2.5	.055	.55
3.0	.06	.6
3.5	.065	.65
4.0	.07	.7
4.5	.075	.75
5.0	.08	.8
6.0	.09	.9
7.0	.1	1.0
8.0	.11	1.1
9.0	.12	1.2
10.0	.13	1.3
11.0	.14	1.4
12.0	.15	1.5
13.0	.16	1.6
14.0	.17	1.7
15.0	.18	1.8
16.0	.19	1.9

Bulk %	P_2O_5 %	Grams P_2O_5 per liter.
17.0	.2	2.0
18.0	.21	2.1
19.0	.22	2.2
20.0	.23	2.3
21.0	.24	2.4
22.0	.25	2.5
23.0	.26	2.6
24.0	.27	2.7
25.0	.28	2.8

GRAVIMETRIC METHODS.

1 100 c. c. filtered urine in beaker.
2 Make up to 200 c. c. with distilled water.
3 Make distinctly acid with acetic acid.
4 Precipitate with solution of uranium nitrate.
5 Filter and wash filter.
6 Burn filter and contents in porcelain crucible.
7 Blast to constant weight.
8 Cool and weigh as UO_2HPO_4.
9 Factor is .1932 for P_2O_5.
10 Multiply by 10 for contents of liter.
1 100 c. c. filtered urine in beaker.
2 Make up to 200 c. c. with distilled water.
3 Precipitate with Ludwig's magnesian mixture.
4 Filter and wash filter.
5 Burn filter and contents in porcelain crucible.
6 Blast to constant weight.
7 Cool and weigh as magnesium pyrophosfate.

8 Factor is .6397 for P_2O_5.
9 Multiply by 10 for contents of a liter.

TITRATION METHODS.

REAGENTS.

1 Uranium nitrate solution.

  1 35.461 grams, pure, dried uranium nitrate. $(UO_2(NO_3)_2 6H_2O)$
  2 Dissolve in small amount acetic acid.
  3 Make up to liter with distilled water.
  4 One c. c. is equivalent to 5 mg. P_2O_5.

2 A solution of sodium acetate and acetic acid.

  1 100 grams NaAc dissolved in distilled water.
  2 100 c. c. 30% acetic acid.
  3 Make up to 1000 c. c. with distilled water.

3 Tincture of cochineal.

  1 Few grams of cochineal.
  2 1 part alcohol, 3 parts water, to 250 c. c.
  3 Digest and decant.

PROCESS.

1 50 c. c. filtered urine in beaker.
2 Add 5 c. c. acetic acid mixture.
3 Few drops cochineal as indicator.
4 Heat to boiling.
5 Titrate while hot with uranium nitrate.
6 End point is a slight permanent green.
7 Factor is .005 for P_2O_5.

8 Multiply No. c. c. uranium solution used by factor and by 20 for amount of P_2O_5 per liter.

FOR EARTHY PHOSFATES.

1 50 to 100 c. c. filtered urine.
2 Make alkaline with NH_4OH and warm.
3 Filter and wash with 1 : 3 NH_4OH.
4 Perforate filter and wash into beaker with a little water and acetic acid.
5 Dissolve in little acetic acid, add water to 50 c. c.
6 Add few drops of cochineal.
7 Titrate hot with uranium nitrate as above.
8 Use same factor.

The difference in results of last two determinations is the phosforic acid in the alkaline phosfates.

ALBUMIN.

QUALITATIVE TESTS.

NITRIC ACID CONTACT.

(Heller's.)

1 1 c. c. urine in tt or plain Purdy tube.
2 Run in urine slowly from pipette.
3 White line of contact indicates albumin.

BOSTON'S METHOD.

1 About 1.5 inches of urine in glass tube.
2 Hold finger over top of tube and wipe dry.
3 Insert tube into bottle of pure HNO_3.

4 Remove finger until acid equals urine.
5 Apply finger and withdraw tube.
6 Hold to light and observe white contact ring if albumin be present.

POTASSIUM FERROCYANIDE AND ACETIC ACID.

1 Test tube half full of filtered urine.
2 Add few drops acetic acid.
3 Add 5% K_4FeCN_6 drop by drop.
4 Cloudy or flaky precipitate indicates albumin.

TANRET'S TEST.

SOLUTION.

1 1.33 gm. $HgCl_2$.
2 3.32 gm. KI.
3 Dissolve in as little water as possible.
4 Add 50 c. c. water and 20 c. c. glacial acetic acid.

PROCESS.

1 Filtered urine in tt or beaker.
2 Add solution drop by drop.
3 A delicate cloud indicates albumin.

SPIEGLER'S TEST.

SOLUTION.

1 8 gm. $HgCl_2$.
2 4 gm. tartaric acid.
3 20 gm. glycerine.
4 200 c. c. distilled water.

PROCESS.

1 Filter the urine.
2 Add few drops acetic acid.
3 Filter again.
4 Place small amount of solution in tt.
5 Run in urine on top of solution.
6 Albumin shows sharp contact ring.
7 Will detect 1 part in 50 000.

JOLLES' MODIFICATION.

SOLUTION.

1 10 gm. $HgCl_2$.
2 20 gm. succinic acid.
3 10 gm. NaCl.
4 500 c. c. water.

Process is the same as Spiegler's Test.

Will detect 1 part in 150 000 to 350 000.

QUANTITATIVE TESTS.

1 10 c. c. filtered urine in graduated Purdy tube.
2 Add 5 c. c. acetic acid and boil.
3 Let stand to settle.
4 Centrifugate to constant volume at 1500 R.P.M.
5 Estimate volume of precipitate.

1 10 c. c. filtered urine in graduated Purdy tube.
2 Add 5 c. c. Esbach's Solution.
3 Mix and warm.
4 Let stand to settle.

5 Centrifugate to constant volume at 1500 R.P.M.
6 Estimate volume of precipitate.

1 Filtered urine in Esbach tube to U.
2 Add Esbach's solution to R.
3 Mix thoroughly and warm.
4 Stand vertically 24 hours.
5 Read amount in grams per liter.

GRAVIMETRIC.

1 100 c. c. filtered urine in beaker.
2 Make acid with acetic acid.
3 Add 30% acetic acid drop by drop, stir and boil.
4 Pass through a dry weighed filter.
5 Wash filter and add drop of HNO_3 and drop of $AgNO_3$.
6 Wash with alcohol and ether.
7 Dry at 120 to 130 C.
8 Weigh and subtract weight of filter.
9 Burn to ash.
10 Weigh the ash and subtract.
11 Result is weight of albumin in 100 c. c.

TITRATION METHOD.

1 20 c. c. filtered urine in small beaker.
2 If alkaline, make acid with acetic acid.
3 Add 30 c. c. distilled water.
4 Add 2 drops 1% aq. sol. true yellow. (Echtgelb.)
5 Titrate with 25% sol. salicylsulphonic acid.
6 End point is a brick red color.

7 Factor is .01006.
8 Multiply by 50 for contents of a liter.

ESBACH'S SOLUTION.

1	Picric acid.	10	grams.
2	Citric acid.	20	"
3	Distilled water. . . .	1000	c. c.

PURDY'S CENTRIFUGAL METHOD.

1 10 c. c. filtered urine in graduated Purdy tube.
2 3 c. c. 10% sol. potassium ferrocyanide.
3 2 c. c. 50% acetic acid.
4 Mix and let stand 10 minutes.
5 Centrifugate 3 min. at 1500 R.P. M.
6 Estimate by following table.

Bulk %	Weight %	Grams per liter.
.25	.005	.05
.5	.01	.1
.75	.016	.16
1.00	.021	.21
1.25	.026	.26
1.5	.031	.31
1.75	.036	.36
2.0	.042	.42
2.75	.057	.57
2.25	.047	.47
2.50	.052	.52
3.0	.063	.63
3.25	.068	.68
3.5	.073	.73
3.75	.078	.78

Bulk %	Weight %	Grams per liter.
4.0	.083	.83
4.25	.089	.89
4.5	.094	.94
4.75	.099	.99
5.0	.104	1.04
5.5	.111	1.11
6.0	.125	1.25
6.5	.135	1.35
7.0	.146	1.46
7.5	.156	1.56
8.0	.167	1.67
8.5	.177	1.77
9.0	.187	1.87
9.5	.198	1.98
10.0	.208	2.08
10.5	.219	2.19
11.0	.229	2.29
11.5	.24	2.4
12.0	.25	2.5
12.5	.26	2.6
13.0	.271	2.71
13.5	.281	2.81
14.0	.292	2.92
14.5	.302	3.02
15.0	.313	3.13
15.5	.323	3.23
16.0	.333	3.33
16.5	.344	3.44
17.0	.354	3.54
17.5	.365	3.65
18.0	.375	3.75

18.5	.385	3.85
19.0	.396	3.96
19.5	.406	4.06
20.0	.417	4.17
20.5	.427	4.27
21.0	.438	4.38
21.5	.448	4.48
22.0	.458	4.58
22.5	.469	4.69
23.0	.479	4.79
23.5	.49	4.9
24.0	.5	5.0
24.5	.51	5.1
25.0	.521	5.21
25.5	.531	5.31
26.0	.542	5.42
26.5	.552	5.52
27.0	.563	5.63

ALBUMIN AND GLOBULIN.

GLOBULIN.

1 50 c. c. filtered urine in beaker.
2 Make alkaline with NH_4OH.
3 Make up to 100 c. c. with distilled water.
4 Add equal amount of sat. neut. sol. ammonium sulfid.
5 Let stand one hour.
6 Filter through weighed filter.
7 Wash with am. sulfid. 50% solution.
8 Dry and weigh.
9 Burn to ash and weigh.
10 Difference is weight of globulin.

ALBUMIN.

1 50 c. c. filtered urine in beaker.
2 Saturate with ammonium sulfid.
3 Let stand one hour.
4 Filter, dry and weigh as total proteids.
5 Subtract weight of globulin determined as above.
6 Remainder is weight of albumin in 50 c. c. urine.
7 Multiply by 20 for contents of liter.

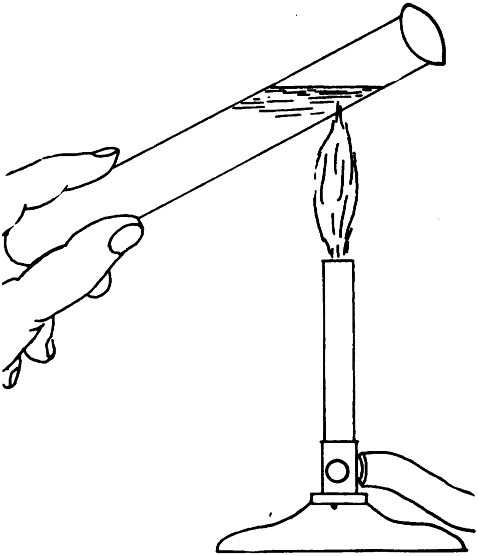


Fig. 21. The Fehling or Haines Qualitative Test.

GLUCOSE.

FEHLING'S METHOD.

SOLUTION NO. I.

1 34.64 grams copper sulfate.
2 500 c. c. distilled water.

SOLUTION NO. II.

1	Rochelle salt	173 grams.
2	KOH	100 "
3	Distilled water	500 c. c.

PROCESS.

1 Place about 2 c. c. sol. No. 1. in tt.
2 Add equal amount of sol. No. II. Mix.
3 Add distilled water till tt. is two-thirds full.
4 Grasp lower end of tt. in left hand.
5 Hold upper end over flame in slanting position.
6 At boiling, add urine from pipette with right hand.
7 If sugar be present, a yellow or red precipitate will form at line of contact.
8 After a moment this precipitate will trickle down through the blue solution.

HAINES' METHOD.

SOLUTION.

1 6 grams copper sulfate.
2 Completely dissolve in 45 c. c. distilled water.
3 Add 45 c. c. pure glycerin.

4 Mix thoroughly.
5 Add 450 c. c. strong solution KOH.

PROCESS.

1 Place about 5 c. c. solution in slender tt.
2 Hold by lower end and boil upper end over flame.
3 Add urine from pipette.
4 In presence of glucose a red or yellow precipitate forms at line of contact.
5 Precipitate soon trickles down through blue liquid.

QUANTITATIVE METHODS.

FEHLING'S.

1 5 c. c. sol. No. I. and 5 c. c. sol. No. II.
2 Place in porcelain evaporating dish.
3 Add 40 c c. distilled water.
4 Bring to a boil on a sand bath.
5 Add urine slowly from a burette, stir.
6 Continue till blue color disappears.
7 If red precipitate obscures the end point,
    1. Filter,
    2 Wash filter with distilled water,
    3 Continue the process.
8 Divide 1000 by No. c. c. urine used and multiply result by .05.
9 Result is grams of glucose per liter.

PURDY'S.

SOLUTION.

1 Pure crystalline copper sulfate. 4.752 grams.

2 Potassium hydroxid, 23.5 grams.
3 Ammonia 350 c. c.
4 Glycerin 38 c. c.
5 Distilled water to 1000 c. c.
6 Dissolve copper sulfate and glycerin in 200 c. c. water with gentle heat.
7 Dissolve KOH in another 200 c. c. of water.
8 Mix, cool, and make up to 1000 c. c. with water.

PROCESS.

1 35 c. c. solution in beaker.
2 Add 70 c. c. distilled water.
3 Bring to boil over sand bath, keep hot.
4 Add urine from burette and stir till blue color disappears.
5 Read amount of urine used.
6 This contained .02 gram of glucose.
7 $\frac{1000}{\text{Amt.}}$ X .20 equals grams of glucose per liter.

FERMENTATION METHOD.

1 Use Einhorn's saccharimeter.
2 Dilute urine to 1% glucose.
3 Fill the measuring tube with urine.
4 Add a pinch of yeast cake.
5 Place in saccharimeter so that long tube is full.
6 Keep in warm place 15 to 24 hours.

7 Read percentage of glucose from graduations on tube.
8 Correct for dilution.

DIFFERENTIAL DENSITY METHOD.

1 Take specific gravity of urine.
2 Place 100 c. c. filtered urine in a flask.
3 Add some fresh yeast.
4 Let stand till fermentation is complete (24 to 48 hr.).
5 Take specific gravity again.
6 Multiply the difference by 230.
7 Result is percentage of sugar.

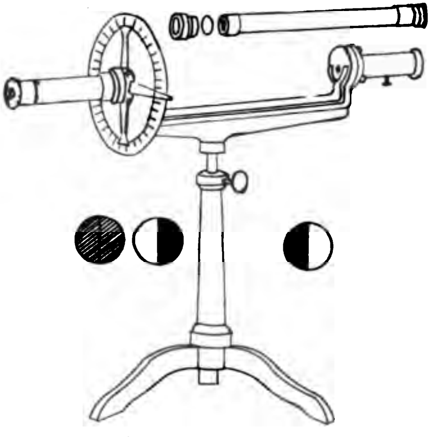


Fig. 22. Saccharimeter.

POLARIZATION.

1 Use fresh clear filtered urine.

2 If urine is colored, take
    1 75 c. c. urine.
    2 25 c. c. 10% sol. PbAc.
    3 Mix and filter.
3 Fill tube of saccharimeter.
4 Place tube in instrument.
5 Adjust vernier and scale.
6 Read per cent of glucose.
7 Correct for dilution with PbAc.

URIC ACID. ($C_5H_4N_4O_3$)

RUHEMANN'S METHOD.

1	Iodin.	1.5	grams.
2	KI.	1.5	“
3	Alcohol.	15.0	c. c.
4	Distilled water.	185.0	“

PROCESS.

1 Use Ruhemann's uricometer tube.
2 Fill to first mark with CS_2.
3 Add Ruhemann's solution to third mark.
4 Add urine slowly, invert and shake.
5 Continue slowly till CS_2 becomes white.
6 Read amount of uric acid in grams per liter.

CAUTION.

Do not hurry the process.
The urine must be slightly acid.

MODIFICATION NO. I.

1 Use a graduated Purdy tube.
2 Run in .5 c. c. CS_2.

3 Add 2 c. c. Ruhemann's solution.
4 Run in urine slowly, invert and shake.
5 Continue slowly till CS_2 becomes white.
6 Subtract 2.5 c. c. from top reading of tube.
7 Estimate by Ruhemann's table.

MODIFICATION NO. II.

1 Place .5 c. c. CS_2 in tt.
2 Add 2 c. c. Ruhemann's solution.
3 Run in urine slowly from a burette.
4 Invert and shake often.
5 Continue slowly till CS_2 is white.
6 Read amount of urine used from burette.
7 Estimate uric acid from Ruhemann's table.

RUHEMANN'S TABLE.

Urine used. c. c.	Uric acid. Grams per liter.
2.6	2.45
2.8	2.15
3.0	1.89
3.2	1.63
3.4	1.38
3.6	1.13
3.8	.94
4.0	.8
4.2	.76
4.4	.71
4.6	.653
4.8	.6
5.0	.55

Urine used. c. c.	Uric acid. Grams per liter.
5.2	.5
5.4	.47
5.6	.44
5.8	.41
6.0	.38
6.2	.35
6.4	.33
6.6	.3
6.8	.28
7.0	.26
7.2	.252
7.4	.249
7.6	.245
7.8	.242
8.0	.238
8.2	.235
8.4	.231
8.6	.228
8.8	.225
9.0	.221
9.2	.218
9.4	.215
9.6	.211
9.8	.208
10.0	.205
10.2	.202
10.4	.199
10.6	.196
10.8	.193
11.0	.190

11.2	.187
11.4	.184
11.6	.181
11.8	.178
12.0	.175

FOLIN'S METHOD.

SOLUTIONS.

1 500 grams ammonium sulfate, and
2 5 grams uranium acetate, dissolved in
3 650 c. c. distilled water.
4 Add 60 c. c. 10% acetic acid.
5 Make up to a liter with distilled water.

An N/20 solution of $KMnO_4$. Make an N/10 solution and dilute.

PROCESS.

1 300 c. c. filtered urine in beaker.
2 Add 75 c. c. uranium acetate solution.
3 Stir and let stand 5 min.
4 Filter and wash filter.
5 Measure 125 c. c. filtrate into two beakers.
6 Add 5 c. c. strong ammonia to each.
7 Mix and set aside till next day.
8 Decant through a filter.
9 Wash precipitate on filter with 10% solution of ammonium sulfate.
10 Pierce filter and wash into beaker with 100 c. c. water.
11 Add 15 c. c. concentrated H_2SO_4.
12 Titrate with N/20 $KMnO_4$ to first blush of red.

13 Factor is .00375.
14 Add a correction of .003 for each 100 c. c. of urine.

HEINTZ'S METHOD.

1 200 c. c. filtered albumin free urine.
2 Add 10 c. c. HCl.
3 Stand in cool place 24 hours.
4 Filter through a dried and weighed filter.
5 Wash with cold distilled water.
6 Dry at 100 C. cool and weigh.
7 Subtract weight of filter.

1 200 c. c. filtered albumin free urine.
2 Treat with Esbach's solution.
3 Stand in cool place 24 hours.
4 Filter through a dried weighed filter.
5 Wash with cold distilled water.
6 Dry at 100 C. cool and weigh.
7 Subtract weight of filter.

PURIN BODIES.

COOK'S METHOD.

1 Use a graduated Purdy tube.
2 10 c. c. filtered urine.
3 1 gram sodium carbonate.
4 One to two c. c. stronger ammonia.
5 Shake until soda is dissolved.
6 Centrifugate the precipitated phosfates.
7 Pour off the clear fluid into another Purdy tube.
8. Add 2 c. c. ammonia and 2 c. c. ammoniated silver nitrate.
9 Mix and let stand few minutes.

10 Centrifugate to constant volume.
11 Multiply the bulk percentage by .001176. This gives the weight in grams in 10 c. c.
12 Multiply the bulk percentage by .1176; the result is in grams per liter.
13 Multiply this result by .9; the result is a close approximation of the uric acid.

AMMONIATED SILVER NITRATE SOLUTION.

1 Dissolve 5 grams $AgNO_3$ in 100 c. c. distilled water.
2 Add ammonia till solution clouds and clears again.

UREA $CO(NH_2)_2$.

RICE'S SOLUTIONS.

1	Bromin.	10 c. c.
2	Potassium bromid.	31 grams.
3	Distilled water.	250 c. c.

1	Sodium hydroxid.	100 grams.
2	Distilled water.	250 c. c.

PROCESS.

1 Use a Doremus or a Doremus-Hinds ureometer.
2 Mix the solutions and water in proportion of 1, 1, and 2.
3 Fill the ureometer and bulb.
4 Run in one c. c. of urine.

5 When gas formation ceases, read the graduations in grams per c. c. of urine used.
6 Multiply by 1000 for grams per liter.

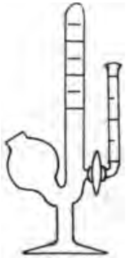


Fig. 23. Doremus-Hinds Ureometer.

METHOD OF FOWLER.

1 Use sodium hypochlorite.
2 Have urine and sodium hypochlorite at same temperature.
3 Very carefully take the specific gravity of both.
4 Take exactly 70 c. c. hypochlorite.
5 Add exactly 10 c. c. urine.
6 After first violent reaction,
7 Shake occasionally for an hour.
8 Multiply specific gravity of hypochlorite by 7.
9 Add specific gravity of urine.
10. Divide by 8.
11. At end of one hour,
12 Bring the mixture to same temperature as at first.
13 Carefully take the specific gravity.

14 Subtract from average at step No. 10.
15 Multiply by .7791 and by 10.
16 Result is grams urea per liter.

TOTAL NITROGEN.

KJELDAHL METHOD OR MODIFICATION.

1 20 c. c. urine.
2 15 c. c. pure sulfuric acid.
3 10 grams potassium sulfate.

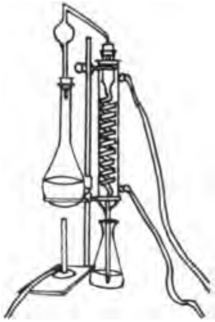


Fig. 24. Kjeldahl Apparatus.

4 1 gram copper sulfate.
5 Place in Jena combustion flask of 250 to 300 c. c. capacity.
6 Boil under hood till fluid is clear blue.
7 Continue heat about half hour longer.
8 Little $KMnO_4$ may be added.
9 Cool, add water in excess and place in long necked liter flask with round bottom.
10 Add talcum powder or granulated zinc.

11 Make alkaline with strong NaOH.
12 Fit flask to Liebig condenser.
13 Fit both tubes with safety bulbs.
14 Place 50 c. c. N/4 sulfuric acid in receiving flask.
15 Immerse end of delivery tube in this acid.
16 Boil slowly till 100 c. c. have passed over.
17 Titrate with N/4 NaOH.
18 Subtract c. c. N/4 NaOH from 50.
19 Multiply remainder by .0035.
20 Result is nitrogen in amount of urine used.
21 Multiply by 50 for amount of nitrogen per liter.

OXALIC ACID.

1 Take 600 c. c. urine.
2 Add small amount alcoholic sol. thymol.
3 Add excess of calcium chlorid and ammonia.
4 Add acetic acid to a weak acid reaction.
5 Let stand 24 hours.
6 Filter and wash.
7 Dissolve precipitate with HCl.
8 Wash filter with distilled water.
10 Make slightly alkaline with ammonia.
11 Stand 24 hours.
12 Filter and wash.
13 Burn filter.
14 Blast to constant weight.
15 Weigh as CaO.
16 Factor is 1.8 for oxalic acid and 2.2858 for calcium oxalate.

THE DIAZO REACTION.

SOLUTIONS.

I.

1. Water, 95 c. c.
2 HCl 5 c. c.
3 Sulphanilic acid 1 gram.

II.

.5% sol. sodium nitrite.

PROCESS.

1 10 to 20 c. c. urine in tt.
2 Add equal volume of sol. No. 1.
3 Shake and mix well.
4 Add 3 to 6 drops sol. No. 2.
5 Shake till froth appears.
6 Make alkaline with NH_4OH.
7 Positive reaction gives a port wine red and red froth.

BILE IN URINE.

IODIN TEST.

1 Place 10 c. c. urine in tt.
2 Overlay with 10% alcoholic sol. iodin.
3 An emerald green line of contact identifies bile.

GMELIN'S TEST.

1 10 to 15 c. c. urine in a conical glass.
2 With long stemmed funnel, underlay with yellow HNO_3.

3 A contact line of yellow, green and violet colors identifies bile.
4 The green and violet line rises the longer the contact.

HAY'S TEST.

1 100 c. c. filtered urine in tall slender beaker.
2 Sprinkle with finely powdered sulfur.
3 Sulfur will sink in presence of bile acids.
4 If it sinks at once, .01% or more.
5 If after gentle shaking, .0025% or more.
6 If it does not sink at all, bile acids are absent.

ACETONE. (CH_3COCH_3)

1 Use 250 to 1000 c. c. fresh urine.
2 Make alkaline with KOH.
3 Shake out with ether to remove diacetic acid.
4 Separate the ether in separatory funnel.
5 Add a little phosforic acid to urine.
6 Distil over about 30 c. c.
7 Apply following tests to distillate.

GUNNING'S TEST.

1 10 c. c. distillate in tt.
2 Add tincture iodin or Lugol's solution.
3 Add ammonia until a black precipitate forms.
4 Black precipitate disappears, leaving
5 A yellow sediment of iodoform.

LIEBEN'S TEST.

1 10 c. c. distillate in tt.
2 Add few drops KOH.

3 Add Lugol's solution and warm.
4 Crystals of iodoform will identify acetone.

LANGE'S TEST.

1 10 c. c. filtered urine in tt.
2 Add 10 drops glacial acetic acid.
3 Add few drops con. aq. sol. sodium nitroprussid.
4 Gently overlay with a little ammonia.
5 A purple ring of contact in a few minutes will identify acetone.

TROMMER'S TEST.

1 10 c. c. filtered urine in tt.
2 Add 2 to 3 c. c. 40% NaOH.
3 Add 10 to 12 drops 10% alcoholic sol.
3 Add 10 to 12 drops 10% alcoholic sol. salicylous acid.
4 Hold tt. by lower end.
5 Heat upper part nearly to boiling 5 min. or more.
6 Heated portion will become purplish red in presence of acetone.

QUANTITATIVE ESTIMATION.

1 50 to 250 c. c. urine in distilling flask.
2 Add a little phosforic acid to prevent foaming.
3 Delivery tube must be immersed in water in receiving flask.
4 Distil till greater part has passed over.

5 Pour distillate into glass stoppered graduated cylinder.
6 Add 15 to 20 c. c. NaOH.
7 Add 20 c. c. triple strength Lugol's Sol.
8 Black precipitate forms followed by yellow.
9 After 10 to 20 min. add 40 to 50 c. c. ether.
10 Shake until ether contains all the iodoform.
11 Separate the ether and evaporate to dryness.
12 Dry over sulfuric acid and weigh.
13 Weight x .147 equals weight of acetone in urine used.

DIACETIC ACID. (CH_3COCH_2COOH)

GERHARDT'S TEST.

1 10 c. c. filtered urine in tt.
2 Add 10% sol. ferric chlorid as long as precipitate forms.
3 Filter.
4 Add more ferric chlorid (Fe_2Cl_6).
5 A Bordeaux red indicates diacetic acid.
6 Color will disappear on boiling.

LINDERMANN'S TEST.

1 10 c. c. filtered urine in tt.
2 Add 5 drops 30% acetic acid.
3 Add 5 drops Lugol's solution.
4 Add 2 or 3 c. c. chloroform.
5 If chloroform remains clear, diacetic acid is present.
6 In absence of diacetic acid, chloroform becomes reddish violet.

Test for Acetone and Diacetic Acid before surgical operations, especially before giving chloroform.

DRUG POISONING.

ACETANILID AND PHENACETIN.

1 Evaporate the urine to one-half volume.
2 Add one-fifth volume HCl.
3 Boil for a few minutes.
4 Extract with ether.
5 Separate the ether.
6 Evaporate ether to dryness.
7 Dissolve residue in water.
8 Take 10 c. c. of solution in tt.
9 Add few c. c. 3% phenol.
10 Add weak sol. chromic acid drop by drop.
11 Fluid becomes red.
12 Add ammonia.
13 Fluid becomes blue.

ANTIPYRIN.

1 10 c. c. filtered urine in tt.
2 Add few drops 10% solution ferric chlorid.
3 Fluid will become dark red.
4 Color will not disappear on boiling.

THE BROMIDES.

1 10 c. c. urine in tt.
2 Add few drops dilute sulfuric acid.
3 Add few drops fuming nitric acid.
4 Add few c. c. chloroform and shake.
5 Chloroform will sink to bottom and become yellow.

IODIN.

1 Same process as for bromides.
2 Chloroform will become pink to reddish violet.
3 To detect traces, add sodium carbonate and reduce volume by evaporation.

URINARY SEDIMENTS.

COLLECTION.

BY CENTRIFUGATION.

1 15 c. c. fresh urine in Purdy tubes.
2 Centrifugate.
3 Pour off urine.
4 Fill again with distilled water.
5 Centrifugate again.
6 Pour off the water.

BY FILTRATION.

1 Place small fine filter paper in funnel.
2 Shake up the whole sample of fresh urine.
3 Filter the whole sample.
4 Examine the sediments in last cubic centimeter.

EXAMINATION.

1 Take sediment from tube or filter with pipette or medicine dropper.
2 Place one drop on slide.
3 Place cover glass.
4 Examine with low power of microscope.

5 Examine particular objects with 1/6 objective.
6 Pay attention to adjustment of light.

TEST OF OLD SAMPLE FOR GLUCOSE.

1 An old sample of urine is strongly acid.
2 Place sediment on microscope.
3 Examine for yeast cells.
4 Make a common cover glass preparation.
5 Stain with methylene blue.
6 Examine for yeast cells.

B. TUBERCULOSIS IN URINE.

1 Add acetic acid, then heat gently.
2 Centrifugate thoroughly.
3 Pour off urine and add water.
4 Centrifugate again.
5 Pour off and add alcohol.
6 Centrifugate again, and pour off alcohol.
7 Make smears of sediment with little white of egg.
8 Dry and fix in flame.
9 Stain with carbol-fuchsin, steaming three to five minutes.
10 Destain in acid alcohol.
11 Wash in water.
12 Soak in alcohol over night to discolor the smegma bacillus.
13 Wash in water.
14 Counter stain with methylene blue.
15 Wash, dry and mount.

AMORPHOUS URATES.

1 5 c. c. turbid urine in tt.
2 Heat gently.
3 If sediment is urates.
4. Urine will become clear.

BLOOD IN URINE.

GUM GUIAC TEST.

1 Urine in small tt.
2 Add three drops tincture gum guiac.
3 Add three drops old turpentine or hydrogen peroxid.
4 Blue color indicates blood.
5 Is a very delicate test.

CHAPTER VI.

BACTERIOLOGY.

PREPARATION OF CULTURE MEDIA.

BOUILLON.

1 1000 c. c. of tap water.
2 Add 3 grams beef extract.
3 Boil till dissolved.
4 Cool and filter.
5 Add,

    1 10 grams peptone.
    2 5 grams NaCl.
    3 5 grams Kali Phos.

6 Boil and test for reaction.
7 Render slightly alkaline with NaOH.
8 Filter into flasks or tubes.
9 Sterilize by fractional sterilization.

To grow B. Tuberculosis, add 6% of glycerine between 6 and 7.

GELATIN.

SOLUTION NO. 1.

1 500 c. c. of tap water.

2 3 grams extract of beef.
3 Boil, cool and filter.

SOLUTION NO. 2.

1 500 c. c. of tap water.
2 10 grams peptone.
3 5 grams NaCl.
4 5 grams Kali Phos.
5 150 grams gelatin.
6 Boil till dissolved.

1 Pour both solutions together.
2 Boil and test reaction.
3 Render slightly alkaline with NaOH.
4 Filter through cotton.
5 Tube—five to ten c. c.
6 Fractional sterilization.
    1 Not too long—30 min.
    2 Not too often—3 times.
    3 Not too hot—100 C.

AGAR-AGAR.

SOLUTION NO. 1.

1 500 c. c. of tap water.
2 3 grams beef extract.
3 Boil, cool and filter.

SOLUTION NO. 2.

1 500 c. c. of tap water.
2 10 grams peptone.
3 5 grams NaCl.
4 5 grams Kali Phos.

5 15 grams agar-agar.
6 Boil till thoroughly dissolved.
1 Pour both solutions together.
2 Make up to 1000 c. c. with water.
3 Boil and test reaction.
4 Render slightly alkaline with NaOH.
5 Filter through cotton into flasks or tubes.
6 Fractional sterilization.

SPECIAL VARIATIONS.

1 To grow B. Tuberculosis, add 6% of glycerin.
2 For gas production, add 2% glucose.
3 For acid production tests, add filtered sol. of litmus.

BLOOD SERUM.

1 Collect fresh blood in large vessel.
2 Permit clot to form.
3 Draw off the serum.
4 Filter.
5 Tube.
6 Coagulate at low heat in slanting position.
7 Sterilize in steam sterilizer.

MILK.

1 Tube fresh milk.
2 Fractional sterilization.

POTATO.

1 Pare good potatoes.
2 Cut into cylinders.

3 Cut cylinders diagonally.
4 Place in test tube slant end up.
5 Cook and sterilize in steam sterilizer.

Ascitic fluid, cystic contents, and urine can be used instead of bouillon. Such mixtures are necessary to the growth of particular bacteria, such as the gonococcus.

ISOLATION.

1 Prepare sterile Petri dish.
2 Liquefy sterile agar in flask or tube.
3 Pour agar in Petri dish.
4 Cover and cool till dish can be held on hand.
5 Inoculate with material to be tested.
6 Give dish rotary motion to mix and spread material.
7 Keep at optimum temperature till colonies develop.
8 Transfer desired colonies to slant agar tubes.

M. A. BARBER'S METHOD.

1 Use microscope with mechanical stage.
2 Use M. A. Barber's instrument.
3 Attach instrument to stage of microscope.
4 Moisten chamber with strips of filter paper saturated with sterile water.
5 Rule a 1 by 2 in. rectangular cover glass into 4 segments with India ink.
6 Rim moist chamber with vaselin or vaselin-paraffin.
7 Place the cover glass.
8 Make hanging drops on segments of cover glass of

1 Material to be examined.
2 Sterile bouillon.
3 Sterile normal salt solution.

9 Use a 1/5 or 1/6 objective.
10 Bring drop of material to be examined into focus.
11 Select bacterium to be isolated.

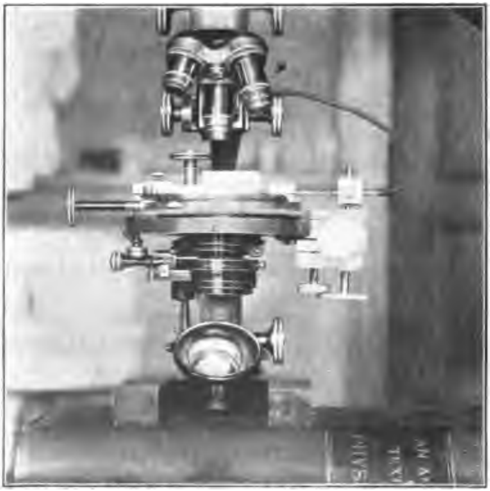


Fig. 25. **Barber's Isolating Apparatus Attached to Microscope.**

12 Bring capillary pipette into focus.
13 Draw in the bacterium.
14 Move the pipette to one of the sterile drops.

15 Expel the bacterium into the sterile drop.
16 Observe development.
17 Transfer to sterile agar tube.

Instrument invented by Dr. M. A. Barber of the University of Kansas.

STAB CULTURE.

1 Agar tube cooled in vertical position.
2 Take culture on platinum wire.
3 Insert wire into agar.
4 Withdraw wire in track of puncture.
5 Stop tube with cotton.

STREAK CULTURE.

1 Agar tube cooled in slanting position.
2 Take culture on platinum loop.
3 Insert loop into tube to water of condensation.
4 Withdraw loop along surface of agar.
5 Stop tube with cotton.

LIQUEFACTION.

1 Gelatin tube cooled in vertical position.
2 Make stab culture as in agar.
3 Grow at room temperature.

GAS PRODUCTION.

IN SOLID MEDIUM.

1 Liquefy tube of glucose agar.
2 Inoculate while liquid but not too hot.
3 Permit to solidify in vertical position.

4 Incubate at 37C. or at optimum temperature.
5 Gas will show as bubbles in 3 or 4 days.

IN LIQUID MEDIUM.

1 Fill fermentation tube with glucose bouillon.
2 Inoculate.
3 Grow at optimum temperature.
4 Gas will collect in the long limb.

TO DETERMINE THE GAS FORMULA.

1 Use a graduated fermentation tube.
2 Fill with glucose bouillon.
3 Inoculate.
4 Develop to the maximum of gas production.
5 Read and record the amount of gas.
6 Run in a strong solution of KOH.
7 When volume is again constant, read and record.
8 Amount remaining in tube represents H.
9 Subtract amount remaining in tube from total reading.
10 Remainder represents CO_2.
11 Express results in a fraction $\frac{H}{CO_2}$.

COUNTING BACTERIA.

1 Pour sterile agar in sterile Petri dish.
2 Inoculate with measured quantity of material, 1 c. c. or less.

3 Grow 3 days or more at optimum temperature.
4 Place dish over marked disc.
  1 Black disc same size as dish.
  2 Ruled in 16 to 32 segments in white ink.
5 Count colonies in segments—Using a reading glass.
6 Take average, multiply by number of segments, and by dilution, if any.

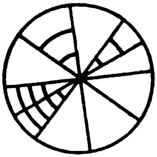


Fig. 26. Ruled Disc For Counting Bacteria.

TO CULTIVATE ANEROBES.

1 Prepare salt mouthed jar with two tight fitting corks.
2 Prepare hydrogen generator with delivery and overflow tubes.
3 Fit delivery tube to one cork of salt mouth jar.
4 Prepare small test tubes of liquefied glucose agar.
5 Inoculate agar tube with garden or barn yard earth and place in jar.
6 Run hydrogen into jar till oxygen is entirely displaced.

7 Bring extra cork to mouth of jar in one hand.
8 Remove perforated cork and quickly insert cork.
9 Incubate at 37 C.

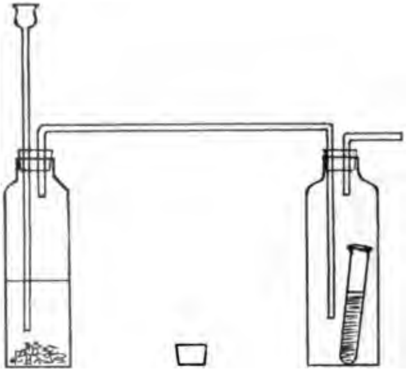


Fig. 27. Apparatus For Anerobic Cultures.

TO ISOLATE AND IDENTIFY SEWAGE BACTERIA.

COLI TYPES.

1 Take 10 c. c. glucose agar.
2 Add four drops 5% phenol.
3 Make light blue with litmus solution.
4 Add 1 c. c. of water to be examined.
5 Incubate at 37 C.
6 Colonies producing gas and red color are of coli type.

7 Can be counted in tube, or in Petri plate.

B. COLI AND B. TYPHOSUS.

METHOD OF REMY.

1 Take of

1	Asparagin	6.00	grams.
2	Oxalic acid	.5	"
3	Lactic acid	.15	"
4	Citric acid	.15	"
5	Disodic phosfate	5.00	"
6	Potassium sulfate	1.25	"
7	Sodium chlorid	2.00	"

2 Pulverize in mortar.
3 Place in 100 c. c. distilled water.
4 Add 30 grams peptone.
5 Boil or heat in autoclave 15 minutes.
6 Add 150 grams gelatin—dissolve.
7 Render slightly alkaline with NaOH.
8 Boil or heat in autoclave at 110 C. 15 minutes.
9 Acidify with N/2 H_2SO_4 till 10 c. c. equal .2 c. c. N/2 NaOH.
This will give .5 c. c. H_2SO_4 to the liter.
10 Place in steam sterilizer 10 minutes.
11 Filter through cotton.
12 Add 2.5 grams magnesium sulfate.
13 Dissolve, tube, sterilize by fractional sterilization.
14 At moment of using add 1 c. c. 35% sol. lactose and .1 c. c. 2.5% sol. phenol. to each tube.

15 Add material to be tested.
16 Grow at room temperature.
17 B. Coli colonies appear yellowish brown:
18 B Typhosus colonies bluish white and small.
19 B. Coli colonies produce gas.

METHOD OF HISS.

FOR USE IN TUBES.

1 5 grams agar-agar.
2 5 grams beef extract.
3 5 grams NaCl.
4 Dissolved in 1000 c. c. water.
5 Add 80 grams gelatin and dissolve.
6 Determine reaction and bring to 1.5% of normal acid by HCl or NaHO.
7 Beat two eggs in 25 c. c. water.
8 Add to medium, boil 45 minutes.
9 Filter through cotton.
10 Add 10 grams glucose.
11 Tube and cool in vertical position.
12 Make stab cultures.
13 B. Typhosus clouds the medium uniformly without streaks or bubbles.

FOR PLATING.

1 10 grams agar-agar.
2 5 grams beef extract.
3 5 grams NaCl.
4 Dissolve in 100 c. c. water.
5 Add 25 grams gelatin and dissolve.
6 Correct reaction to 2% of normal acid.
7 Clear with egg.

8 Filter through cotton.
9 Add 10 grams glucose.
10 B. Typhosus alone produces thread-forming colonies.

PIORKOWSKI'S METHOD.

1 1000 c. c. urine two days old.
2 5 grams peptone.
3 33 grams gelatin.
4 Dissolve and filter.
5 Fractional sterilization.
6 Use in tube or plates.
7 Inoculate.
8 Keep at 22 C.
9 Colonies of B. Typhosus appear radiated and filamentous.
10 Colonies of B. Coli are round, yellowish and sharply defined.

METHOD OF ADAMI AND CHAPIN.

1 2 liters of suspected water.
2 Place in 2 one liter flasks or bottles.
3 Add 25 c. c. nutrient bouillon to each.
4 Incubate 18 to 24 hours at 37 C.
5 Prepare a number of glass tubes .5 meter long.
6 Seal one end in flame and file around 3 or 4 centimeters from sealed end.
7 Transfer 10 c. c. of culture in water to each tube.
8 Add typhoid or typhoid immune serum in amounts to make dilutions 1:60, 1:100, 1:150, 1:200.

9 Aggulination will occur in from 15 min. to 2 to 5 hours.
10 When agglutination is complete, break off tube at file mark.
11 Wash agglutinated sediment with distilled water.
12 Permit to settle.
13 Decant.
14 Inoculate various media with loops of the sediment.

DRIGALSKI-CONRADI METHOD.

HAGEMANN'S MODIFICATION.

1 600 c. c. water.
2 10 grams beef extract.
3 10 grams peptone.
4 10 grams NaCl.
5 Boil in salt water bath till 100 c. c. evaporates.
6 Add 500 c. c. fresh milk.
7 Boil and add 10 grams agar.
8 Boil till agar is dissolved.
9 Filter through cotton.
10 Divide up into 200 c. c. flasks.
11 Sterilize short time.
12 In using, melt in water bath.
13 Make slightly alkaline with NaOH.
14 Add 20 c. c. litmus solution.
15 Add three drops 1% alcoholic sol. crystal violet.
16 Pour into Petri plates and use as follows:

17 Use a glass rod, bent at end, with knob on end.
18 Dip knob in suspected material.
19 Smear over surface of cool Petri plates.
20 Colonies of B. Coli in 14 to 16 hours, red and opaque.
21 Colonies of B. Typhosus, blue or violet, transparent and drop like.

LOEFFLER'S MALACHITE GREEN.

1 Prepare ordinary nutrient agar.
2 Color with malachite green.
3 Make Petri plates.
4 B. Coli is inhibited while B. Typhosus grows.
5 B. Typhosus produces a yellow color.
6 Malachite green varies in strength and samples must be tested separately.

CAFFEIN ENRICHMENT METHOD.

FOR ISOLATING B. TYPHOSUS FROM FECES.

1 Prepare liter of bouillon, sterilize and bottle.
2 Enriching fluid.
  1 100 c. c. of the bouillon.
  2 Add NaOH to 2.7 c. c. less than required for phenolphthalein "red point."
  3 Sterilize ten minutes, and cool.
  4 Add 105 c. c. 1.2% sol. caffein in cold distilled water.
  5 Add 1.4 c. c. .1% sol. crystal violet, dissolved cold.
3 Preparation of feces.

- 1 Thin stool,
    - 1 Permit to settle.
    - 2 Take .8 to .9 c. c. of upper portion.
    - 3 Add to the enriching fluid.
- 2 Semi-fluid stool.
    - 1 One part of feces.
    - 2 One part 1.2% sol. caffein.
    - 3 Rub up in mortar.
    - 4 Filter through sterile cotton.
    - Place .8 to .9 c. c. filtrate in enriching fluid.
- 3 Thick feces.
    - 1 One part feces.
    - 2 Two parts 1.2% sol. caffein.
    - 3 Proceed as above.

4 Shake thoroughly, and incubate at 37 C.
5 Examine a wet preparation or hanging drop.
6 Make Drigalski-Conradi plates.

THE WIDAL REACTION.

1 Stock culture of B. Typhosus.
2 Fresh 24 hour cultures on agar or in bouillon.
3 A drop or two of patient's blood on a slide.
4 Make serum by adding drop of distilled water to blood.
5 Rim the concavity of a hollow slide with vaselin.
6 Place loop of distilled water on cover glass.
7 Transfer loop of serum to cover glass.
8 Transfer loop of culture to cover glass.

9 Invert concavity of slide over cover glass.
10 Examine with 1/4, 1/5, or 1/6 objective.
11 Motility ceases and clumping occurs in 15 min. to 1 hour if reaction is positive.

MOTILITY.

THE WET PREPARATION.

1 Place drop of sterile water in center of slide.
2 Inoculate with culture.
3 Place cover glass.
4 Examine with 1/4, 1/5, or 1/6 objective.

THE HANGING DROP PREPARATION.

1 Place hollow ground slide with concavity up.
2 Rim concavity with small amount of vaselin.
3 Place cover glass on glass plate.
4 Place loop of sterile water in center of cover glass.
5 Inoculate drop with culture.
6 Invert slide over cover glass.
7 Examine with 1/4, 1/5, or 1/6 objective.

STAINING BACTERIA.

THE COMMON COVER GLASS PREPARATION.

1 Cover glass in wire forceps.
2 Spread material on cover glass.
3 Dry in air.
4 Fix—pass three times through flame.
5 Cover with stain—one to five minutes.
    1 Methylene blue, or
    2 Gentian violet, or
    3 Fuchsin.

6 Wash in water.
7 Dry.
8 Mount in balsam.
9 Examine with oil immersion objective.

THE ZIEHL-NEELSON METHOD.

1 Cover glass in forceps.
2 Spread material on cover glass—sputum.
3 Dry in air.
4 Fix—three times through flame.
5 Cover with carbol-fuchsin.
6 Heat to steaming three to five minutes—do not dry.
7 Wash in water.

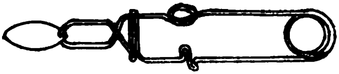


Fig. 28. Cover Glass in Novy's Forceps.

8 Destain in acid alcohol—3% HCl in 70% alcohol.
9 Wash in water.
10 Counterstain in methylene blue one minute or less.
11 Wash in water.
12 Dry, and mount in balsam.
13 B. Tuberculosis stains red, everything else blue.

GRAM'S METHOD.

1 Prepare fresh alilin water. 1 c. c. anilin oil, 50 c. c. water, shake to emulsion and filter.

2 Cover glass of material in forceps.
3 Fix—three times through flame.
4 Fill with anilin water.
5 Add gentian violet stain.
6 Heat to steaming five to ten minutes.
7 Wash in water.
8 Place in Gram's solution.

1	Iodin.	1 gram.
2	KI.	2 "
3	Distilled water.	300 c. c.

9 Wash in water.
10 Destain in 95% alcohol till no more stain comes away.
11 Counterstain with Bismark brown.
12 Wash, dry and mount.
13 Gram positive bacteria stain blue to black.
14 All else stains brown.

STAINING CAPSULES.

BUERGER'S METHOD.

1 Mix sputum, blood serum and egg albumin solution.
2 Make smears—let edges dry.
3 Cover with Muller's fluid—warm gently 3 seconds.
4 Rinse in water.
5 Flush with alcohol.
6 Stain with tincture iodin 1 to 2 minutes.
7 Wash in alcohol and dry.
8 Stain with anilin water gentian violet 3 seconds.
9 Wash in 2% sol. NaCl.

10 Mount in sol. NaCl and examine.

VABIATION AFTER 7.

8 Stain by Gram's method.
9 Counter stain with aqueous sol. fuchsin.
10 Mount in water.

SMITH'S METHOD.

1 Make thin smears.
2 Dry and fix.
3 Stain with anilin water gentian violet a few seconds—warm to steaming.
4 Rinse in water.
5 Stain by Gram 30 seconds.
6 Wash in 95% alcohol.
7 Wash in alcohol and ether, equal parts.
8 Stain in eosin.
9 Stain with Loeffler's methylene blue and heat to steaming.
10 Wash in water.
11 Rinse quickly in absolute alcohol.
12 Apply xylol 30 seconds.
13 Mount in balsam.

STAINING FLAGELLA.

METHOD OF LOEFFLER.

1 Perfectly clean cover glass.
2 Young culture from water of condensation, or from bouillon.
3 Let the drop spread on cover glass.
4 Dry and fix.
5 Cover with the following mordant:

    1 Tannic acid, 20% sol.........10 c. c.
    2 Sat. Sol. ferrous sulfate...... 5 c. c.
    3 Sat. Sol. alcoholic fuchsin..... 1 c. c.
6 Warm slightly one to five minutes.
7 Wash in water.
8 Stain with anilin water fuchsin or carbol fuchsin.
9 Wash in water.
10 Dry.
11 Mount in balsam.

METHOD OF VAN ERMENGEM.

1 Make cover glass preparation as before.
2 Dry and fix.
3 Cover with the following mordant.
    1 Osmic acid, 2% sol...........1 part.
    2 Tannic acid, 10 to 25% sol...2 parts.
3 Let stand 30 min. at room temperature, or
4 Five minutes at 50 to 60 C.
5 Wash carefully in distilled water, then in alcohol.
6 Place in .25% to .50% sol. $AgNO_3$ a few seconds.
7 Transfer to following sol.
    1 Gallic acid............... 5 grams.
    2 Tannic acid............. 3 "
    3 Fused KAc..............10 "
    4 Distilled water...........350 c. c.
8 After a few seconds return to silver solution.
9 Keep moving till solution turns brown or black.

10 Wash in distilled water.
11 Dry and mount in balsam.

STAINING SPORES.

1 Use the Ziehl-Neelson method.
2 Give more time to the carbol-fuchsin stain.

EXAMINATION OF PUS.

1 Make smear on slide.
2 Dry, and fix in flame.
3 Stain with methylene blue 3 to 5 minutes.
4 Wash in water.
5 Dry—mount in balsam.
6 Examine pus with 1/5 to 1/6 objective.
7 Examine for bacteria with oil immersion objective.

METHOD FOR GONORRHOEA.

1 Smear veneral pus on slide.
2 Dry and fix in flame.
3 Stain with methylene blue three to five minutes.
4 Wash in water.
5 Dry and mount in balsam.
6 Examine with oil immersion objective.
7 Gonoccoci are found in pus cells.
8 For confirmation, stain by Gram. Gonococci will be destained and take the Bismark brown.

EXAMINATION OF DIPHTHERIA.

1 Take to the bedside,

   1 Sterile swab in sterile tube.
   2 Slant tubes of agar or blood serum.
   3 Glass slides.
2 Take material from throat on swab.
3 Make smears on slides.
4 Roll the swab over the surface of culture media in tubes.

IN THE LABORATORY.

1 Fix the smears in flame.
2 Stain with Loeffler's alkaline methylene blue.
3 Wash in water.
4 Dry and mount.
5 Examine with oil immersion lens.

1 Incubate the tubes at 37 C. 6 to 12 hours.
2 Make common cover glass preparations at end of 6 and 12 hours.
3 Stain with Loeffler's alkaline methylene blue.

NEISSER'S STAIN FOR DIPHTHERIA.

1 Methylene blue. . . . . 1 gram.
2 Alcohol. . . . . 20 c. c.
3 Distilled water. . . . . .950 c. c.
4 Glacial acetic acid. . . . . 50 c. c.
5 Stain one to three seconds.
6 Wash.
7 Stain in Bismark brown 3 to 5 seconds.
8 Wash, dry and mount.

STAINING TREPONEMA PALLIDUM.

THE STAIN.

1 12 parts Giemsa's eosin solution.

    1 2.5 c. c. 1% eosin.
    2 500 c. c. water.
2 3 parts azur No. I. (1 :1000 aq. sol.)
3 3 parts azur No. II. (.8:1000 aq. sol.)
4 Keep separate and mix fresh.

PROCESS.

1 Make thin smears on cover glass.
2 Dry in air.
3 Fix in alcohol.
4 Immerse in stain 16 to 24 hours.
5 Wash in water.
6 Dry and mount.

ANIMAL INOCULATION.

1 Prepare sterile hypodermic syringe.
2 Prepare a 24 hour bouillon culture.
3 Clip and shave the part of the animal to be inoculated.
4 Wash place of inoculation with soap.
5 Wash again with alcohol and $HgCl_2$
6 Draw a measured amount of culture into syringe.
7 For subcutaneous inoculation,
    1 Pinch up skin between thumb and finger.
    2 Insert needle in elevated fold.
    3 Slowly inject the culture.
8 For intra peritoneal inoculation:
    1 Pinch up the abdominal wall.
    2 Insert needle in fold.
    3 Inject the culture.

POST MORTEM BACTERIOLOGY.

1 Prepare a number of slant agar tubes.
2 Have sterile knives and plantinum loops.
3 Have alcohol lamp or Bunsen burner.
4 Make a culture from seat of inoculation.
5 Open the heart and make culture from heart's blood.
6 Cut into liver, insert sterile platinum loop, and make culture.
7 Cut into spleen and make culture in same manner.
8 Make cultures from any abscesses or particularly inflamed areas.
9 Always sterilize loop between each operation.

THE OPSONIC INDEX.

THE EMULSION OF BACTERIA.

1 12 to 24 hour agar slant culture.
2 Introduce .85% salt solution.
3 Wash up and down with pipette.
4 Use 1.5% salt solution for T. B. and gonococcus.

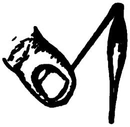


Fig. 29. Drawing Blood From Finger.

WASHED BLOOD CORPUSCLES.

1 Prepare
  1 1.5% sodium citrate solution.

    2 A special long blood pipette.
    3 Two blood tubes for centrifuge.
2 Fill blood tube .75 full with citrate solution.
3 Wrap finger so as to congest the end.
4 Puncture finger and draw blood into citrate sol.
5 Mix blood and citrate solution.
6 Balance tubes and centrifugate.
7 Remove supernatant solution.
8 Wash sedimented corpuscles with .85% salt solution.
9 Centrifugate and remove salt solution.

THE SERUM.

1 Draw blood into a tube or blood pipette.
2 Incubate 15 to 30 minutes.
3 If serum has not separated, centrifugate.

THE MIX.

1 Use opsonizing pipette.
2 Draw in equal parts,

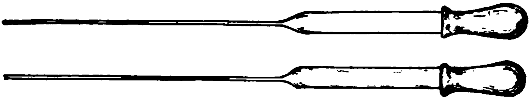


Fig. 30. The Mixing Pipette.

    1 Washed blood corpuscles.
    2 Bacterial emulsion.
    3 Blood serum.
    4 Keep separate by air space.
3 Expel on a slide.
4 Mix by alternate suction and expulsion.

5 Take up into pipette.
6 Seal end of pipette in flame.
7 Incubate 15 minutes.

THE SMEAR.

1 Break point off pipette.
2 Expel contents on end of slide.
3 Make "blood smear."

STAINING.

1 Dry the smear in the air. Stain by Wright's method.
2 Fix the smear in flame.
    1 Stain by Ziehl-Neelson method for T. B.

COUNTING.

1 Place slide on microscope.
2 Use oil immersion lens.
3 Count and record bacteria found in each neutrophile.
4 Take the average.

THE INDEX.

1 Determine this average in the patient.
    1 Use normal washed leucocytes.
    2 Use patient's blood serum.
2 Determine this average in normal blood.
3 Then $\frac{\text{average in patient}}{\text{average in normal blood}}$ is index of patient.

AN EXAMPLE.

1 The average in a patient is found to be 3.
2 The average in normal blood is found to be 4.
3 Then the opsonic index of the patient is 3/4.

GRAM POSITIVE BACTERIA.

Staphylococcus pyogenes aureus.
Streptococcus pyogenes.
Micrococcus lanceolatus.
Micrococcus tetragenus.
Bacillus of diphtheria.
Bacillus of tuberculosis.
Bacillus of leprosy.
Bacillus of anthrax.
Bacillus of tetanus.
Bacillus aerogenes capsulatus.
Actinomyces.

GRAM NEGATIVE BACTERIA.

Gonococcus.
Diplococcus intracellularis meningitidis.
Micrococcus melitensis.
Bacillus of Ducrey.
Bacillus of Shiga.
Bacillus typhosus.
Bacillus coli communis.
Bacillus pyocyaneus.
Bacillus influenzae.
Bacillus of bubonic plague.
Bacillus mallei.
Bacillus of Friedlander.
Spirillum of Asiatic cholera.

Spirillum cholerae nostras.
Spirillum of relapsing fever.

LIST OF GERMICIDES.

Methyl violet (pyoktanin)	1 : 2 000 000—1 : 5000.
Formalin	1 : 25 000—1 : 5000.
Hydrogen peroxid	1 : 20 000.
Bichlorid of mercury	1 : 14 300.
Nitrate of silver	1 : 12 500.
Creolin	1 : 5000—1 : 200.
Chromic acid	1 : 5000.
Thymol	1 : 1340.
Salicylic acid	1 : 1000.
Potassium bichromate	1 : 909.
Trikresol	1 : 1000—1 : 500.
Zinc chlorid	1 : 526.
Potassium permanganate	1 : 285.
Nitrate of lead	1 : 277.
Izal	1 : 200.
Boracic acid	1 : 143.
Chloral hydrate	1 : 107.
Ferrous sulfate	1 : 200—1 : 90.
Calcium chlorid	1 : 25.
Creosote	1 : 20.
Phenol	1 : 50—1 : 20.
Alcohol	1 : 10.

BURGESS' TABLE.

Biniodid of mercury	1 : 5000.
Bichlorid of mercury	1 : 2000.
Chlorinated lime	1 : 100.
Formaldehyd	1 : 40.

Lysol	1 : 20.
Phenol	1 : 20.

METHYL VIOLET.

	Inhibits.	Kills.
Anthrax bacillus	1 : 70 000.	1 : 5000.
Diphtheria	1 : 10 000.	1 : 2000.
Glanders	1 : 2500.	1 : 150.
Typhoid	1 : 2500.	1 : 500.
Cholera	1 : 30 000.	1 : 1000.

APPENDIX.

TABLE OF INTERNATIONAL ATOMIC WEIGHTS.

1905.

Element.	Symbol.	O=16	H=1
Aluminum	Al	27.1	26.9
Antimony	Sb	120.2	119.3
Argon	A	39.9	39.6
Arsenic	As	75.0	74.4
Barium	Ba	137.4	136.4
Bismuth	Bi	208.5	206.9
Boron	B	11.0	10.9
Bromine	Br	79.96	79.36
Cadmium	Cd	112.4	111.6
Caesium	Cs	132.9	131.9
Calcium	Ca	40.1	39.7
Carbon	C	12.0	11.91
Cerium	Ce	140.25	139.2
Chlorine	Cl	35.45	35.18
Chromium	Cr	52.1	51.7
Cobalt	Co	59.0	58.55
Columbium	Cb	94.0	93.3
Copper	Cu	63.6	63.1
Erbium	Er	166.0	164.8

Element.	Symbol.	O=16	H=1
Fluorine	F	19.0	18.9
Gadolinium	Gd	156.0	154.8
Gallium	Ga	70.0	69.5
Germanium	Ge	72.5	72.0
Glucinum	Gl	9.1	9.03
Gold	Ap	197.2	195.7
Helium	He	4.0	4.0
Hydrogen	H	1.008	1.0
Indium	In	115.0	114.1
Iodin	I	126.97	126.01
Iridium	Ir	193.0	191.5
Iron	Fe	55.9	55.5
Krypton	Kr	81.8	81.2
Lanthanum	La	138.9	137.9
Lead	Pb	206.9	205.35
Lithium	Li	7.03	6.98
Magnesium	Mg	24.36	24.18
Manganese	Mn	55.0	54.6
Mercury	Hg	200.0	198.5
Molybdenum	Mo	96.0	95.3
Neodymium	Nd	143.6	142.5
Neon	Ne	20.0	19.9
Nickel	Ni	58.7	58.3
Nitrogen	N	14.04	13.93
Osmium	Os	191.0	189.6
Oxygen	O	16.0	15.88
Palladium	Pd	106.5	105.7
Phosphorus	P	31.0	30.77
Platinum	Pt	194.8	193.3
Potassium	K	39.15	38.85
Praseodymium	Pr	140.5	139.4

LOFFLER'S METHYLENE BLUE.

1	Sat. alcoholic sol. methylene blue	30 c. c.
2	Sol. KOH 1 : 10 000	100 c. c.

ZIEHL'S SOLUTION.

(Carbol-Fuchsin.)

1	Basic Fuchsin	1 gram.
2	Absolute alcohol	10 c. c.
3	5% sol. phenol	100 c. c.

BISMARCK BROWN.

1	Bismark brown	1 gram.
2	Distilled water (boiling)	500 c. c.

ROMANOWSKY'S STAIN.

SOLUTION A.

1	Methylene blue	2 grams.
2	Sodium bicarbonate	9 "
3	Distilled water	25 to 30 c. c.

4 Mix the powders and add water slowly.
5 Cover and steam in sterilizer 75 minutes.
6 Wash residue with water.
7 Add 10 c. c. 4% sol. NaOH and shake.
8 Extract with chloroform and evaporate to dryness.
9 Slowly add 150 c. c. methyl alcohol.

SOLUTION B.

Sat. methyl alcohol sol. watery yellow eosin.

THE STAIN.

1	Solution A	66 c. c.

2 Methyl alcohol. . . . . .33 c. c.
3 Solution B. . . . . .1 to 1.5 c. c.
4 Methylene blue. . . . . .05 to .15 grams.
Especially good in malaria

WRIGHT'S BLOOD STAIN.

1 1.5% sol. sodium bicarbonate in Erlenmeyer flask.
2 Add 1% of methylene blue.
3 Place in steam sterilizer one hour.
4 Remove and cool.
5 Pour into large flask.
6 Add slowly 1 : 1000 sol. yellow watery eosin.
7 Shake often and continue to a purple color, and metallic luster on top.
8 Approximately one part blue to 5 eosin sol.
9 Filter, dry and powder.
10 Place .3 gram in 100 c. c. methyl alcohol.
11 Filter and add 25 c. c. methyl alcohol.
12 Stain is stable, must be kept well corked.

JENNER'S STAIN.

1 1.25% aq. sol. yellow eosin.
2 1% aq. sol. methylene blue.
3 Place equal parts in broad dish.
4 Mix, cover and let stand 24 hours.
5 Filter and dry.
6 Pulverize and wash in distilled water.
7 Shake in flask and filter.
8 Dry and powder.
9 Dissolve .5 gram in 100 c. c. methyl alcohol.

EHRLICH'S TRIPLE STAIN.

1 Saturated aq. sol. orange G........6 c. c.
2 Saturated aq. sol. acid fuchsin......4 c. c.
3 Add slowly and shake, sat. aq. sol. methyl green. . . ..6.6 c. c.
4 Add glycerin. . . .. 5 c. c.
5 Absolute alcohol. . ...10 c. c.
6 Water . . ..15 c. c.
7 Shake well and let stand 24 hours.
8 Do not filter.
9 Do not mix, shake or stir up when using.

PICRO-CARMIN.

1 Ammonium hydrate. . ... 5 c. c.
2 Distilled water. . ...50 c. c.
3 Carmin. . . .. 1 gram.
4 Dissolve and add sat. aq. sol. picric acid. . . ..50 c. c.
5 Expose to light and air two days.
6 Filter.

SAFRANIN.

1 Safranin. . . .. 1 gram.
2 Distilled water. . . ..100 c. c.

METHYL GREEN.

1 Methyl green. . ... 2 grams.
2 Distilled water. . . ..100 c. c.

VAN GIESON'S PICRO-FUCHSIN.

1 Sat. aq. sol. picric acid...........100 c. c.
2 1% aq. sol. acid fuchsin.......... 5 c. c.

SOLUTIONS AND REAGENTS.

LUGOL'S SOLUTION.

Iodin.	1.8	gram.
KI.	1.2	"
Water.	30	c. c.

LUDWIG'S MAGNESIUM MIXTURE.

1	Cryst. mag. chlorid.	100	grams.
2	Water.	1000	c. c.
3	Ammonia to a strong odor.		
4	Ammonium chlorid till all precipitate is dissolved.		

MULLER'S FLUID.

1	Potassium bichromate.	2.5	gram.
2	Sodium sulfate.	1.0	"
3	Distilled water.	100	c. c.

BLOOD DILUTING SOLUTIONS.

HAYEM'S SOLUTION.

1	Mercury bichlorid.	.5	gram.
2	Sodium sulfate.	2.0	"
3	Sodium chlorid.	5.0	"
4	Distilled water.	200.0	c. c.

TOISSON'S MIXTURE.

1	Methyl violet.	.025	gram.
2	Sodium sulfate.	8.0	"
3	Sodium chlorid.	1.0	"
4	Pure glycerin.	30.0	c. c.
5	Distilled water.	160.0	"

OFFICIAL DILUTE HCL.

1 100 grams pure HCl. (31.9%.)
2 219 c. c. distilled water.
3 Contains 10% HCl.

TABLETS.

The tablets described in Water Examination, Chapter IV. are manufactured for the U. S. Geological Survey by the Kremers-Urban Co., of Milwaukee, Wis.

NORMAL AND DECINORMAL SOLUTIONS.

CALCULATED TO OXYGEN AS 16.

Reagent.	Molecular weight.	Grams per liter. N.	N/10
Oxalic Acid ($H_2C_2O_4.2H_2O$)	126.00	63.00	6.300
Sulfuric Acid (H_2SO_4)	98.06	49.03	4.903
Hydrochloric Acid (HCl)	36.45	36.45	3.645
Sodium Carbonate (Na_2CO_3)	106.10	53.05	5.305
Sodium Hydroxid (NaOH)	40.05	40.05	4.005
Potassium Hyroxid (KOH)	56.15	56.15	5.615
Sodium Chlorid (NaCl)	58.5	58.5	5.85
Potassium Permanganate ($KMnO_4$)	158.15	31.63	3.163
Potassium Dichromate ($K_2Cr_2O_7$)	294.5	49.08	4.908
Iodin (I)	126.97	126.97	12.697

Sodium Thiosulfate ($Na_2S_2O_3.5H_2O$)	248.22	248.22	24.822
Silver Nitrate ($AgNO_3$)	169.97	169.97	16.997
Potassium Sulfocyanate ($KSCN$),	97.25	97.25	9.725
Barium Hydroxid ($Ba(OH)_2$)	171.416	85.708	8.57

STANDARDIZING NORMAL AND DECINORMAL SOLUTIONS.

Sulfuric and hydrochloric normals and decinormals are standardized by titration against normal and decinormal carbonates and hydroxids.

They are volumetrically equivalent to oxalic acid.

The standard carbonate is sodium carbonate.

Normal and decinormal sulfuric acid are also standardized by the precipitation of barium sulfate.

The alkalies are standardized by titration against the normal and decinormal acids.

Potassium permanganate is standardized by titration against oxalic acid.

Silver nitrate is standardized by titration against sodium chlorid.

INDICATORS.

1 Litmus solution.
    1 Exhaust powdered litmus with boiling alcohol.

    2 Treat with equal weight of cold water.
    3 Extract with boiling water, 5 times its weight.
    4 Filter.
2 Phenolphthalein.
    1 1 gram in 100 c. c. dilute alcohol.
    2 Colorless in presence of acids.
    3 Purplish red by alkaline hydroxids and carbonates.
    4 Not colored by bicarbonates.
    5 Not suitable for ammonia or bicarbonates.
3 Methyl Orange.
    1 1 gram methyl orange.
    2 Dissolve in 1000 c. c. water.
    3 Yellow in presence of alkalies.
    4 Crimson in presence of mineral acids.
    5 Not affected by carbonic acid.
4 Rosolic Acid.
    1 1 gram in 10 c. c. alcohol.
    2 Add 90 c. c. water.
    3 Used for the ammonia compounds.
    4 Violet red with alkalies.
    5 Yellow with acids.
5 Ferric Ammonium Sulfate.
    1 10 grams in 100 c. c. water.
    2 Used with potassium sulfocyanate.
    3 End point a reddish brown tint.
6 Potassium Chromate.
    1 1 gram in 10 c. c. water.
    2 Used with silver nitrate.

7 Potassium Dichromate.
  1 10 grams in 100 c. c. water.
  2 Used with silver nitrate.

8 Starch Test Solution.
  1 1 gram in 10 c. c. cold water.
  2 Add 200 c. c. boiling water and stir.
  3 Used with iodin and sodium thiosulfate.
  4 Iodin colors starch blue.
  5 Sodium thiosulfate decolorizes the iodin blue starch.

DECINORMAL EQUVILENTS.

OXALIC ACID.

One c. c. decinormal oxalic acid is equivalent to

	Gram.
Ammonia.	.0017064
Calcium Hydroxid.	.0037058
Potassium Hydroxid.	.0056158
Potassium Permanganate. . .	.003163
Sodium Hydroxid.	.004005

SULFURIC ACID.

One c. c. decinormal sulfuric acid is equivalent to

	Gram.
Ammonia.	.0017064
Ammonium Carbonate.	.0048072
Calcium Carbonate.	.005005
Potassium Carbonate.	.006915
Potassium Hydroxid.	.0056158

Sodium Carbonate.	.005305
Sodium Hydroxid.	.004005

HYDROCHLORIC ACID.

Hydrochloric acid has the same equivalents as sulfuric.

POTASSIUM HYDROXID, SODIUM HYDROXID SODIUM CARBONATE.

One c. c. decinormal solution of either is equivalent to

	Gram.
Acetic Acid.	.0060032
Hydrochloric Acid.	.0036458
Lactic Acid.	.0090048
Nitric Acid.	.0063048
Oxalic Acid.	.0063
Phosforic Acid with Phenolphthalein.	.0049012
Phosforic Acid with methyl orange.	.0098024
Sulfuric Acid.	.004903
Tartaric Acid.	.0075024

SILVER NITRATE.

One c. c. decinormal silver nitrate is equivalent to

	Gram.
Ammonium Chlorid.	.0053522
Hydrocyanic Acid with indicator.	.0027048

Hydrocyanic Acid to first precipitate	.0054096
Hydriodic Acid	.0127978
Potassium Cyanid with indicator	.006519
Potassium Cyanid to first precipitate	.013038
Potassium Iodid	.016612
Potassium Sulfocyanate	.009725
Sodium Chlorid	.00585

POTASSIUM SULFOCYANATE.

One c. c. decinormal potassium sulfocyanate is equivalent to

	Gram.
Silver	.010793
Silver Nitrate	.016997

SODIUM CHLORID.

One c. c. decinormal sodium chlorid is equivalent to

	Gram.
Silver	.010793
Silver Nitrate	.016997
Silver Oxid	.011593

POTASSIUM PERMANGANATE.

One c. c. decinormal potassium permanganate is equivalent to

	Gram.
Iron in ferrous compounds	.00559
Oxygen	.0008

SODIUM THIOSULFATE.

One c. c. decinormal sodium thiosulfate is equivalent to

	Gram.
Bromin.	.007996
Chlorin.	.003545
Iodin.	.012697
Iron in ferric salts.	.00559

INDEX

- Abortion. 41
- Acetanilid. 142
- Acetic acid in feces.... 60
- Acetone in urine........139

Acetone:
- Gunning's test. 139
- Lange's test. 140
- Lieben's test. 139
- Quantitative estimation 140
- Trommer's test 140
- When test for........142

Acids:
- Acetic. 49, 60
- Benzoic in milk...... 92
- Bile acids.. 61
- Butyric. 61
- Fatty. 51, 60
- Formic. 60
- Free HCl. 42
- Free in gastric contents. 42
- Hydrochloric. 49, 50
- Lactic. 44, 51
- Oxalic. 137
- Phosforic. 113, 117
- Sulfuric. . ..75, 80, 110, 111
- Uric. 129
- Valerianic. 61
- Acetic acid. 49, 60
  - Adami & Chapin......157
- Added water in milk.... 90
  - Immersion refractometer Meth. 90
- Agar-agar. 147
- Albumin. 124
- Albumin and globulin...123
- Albumin in transudates.. 34
- **Albumin in Urine.**
  - Qualitative tests. 117, 118, 119
    - Boston's method. ...117
    - Jolle's test. 119
    - Nitric acid contact..117
    - Potassium ferrocyanid and acetic acid. 118
    - Spiegler's test. 118
    - Tanret's test. 118
  - Quantitative estimation. 119
    - Esbach's method. . 119
    - Gravimetric method..120
    - Purdy's method.....121
    - Titration method...120
- **Alkalinity**
  - Of blood. 21
  - Of water............ 73
  - Sodium acid sulfate method. 73
  - Titration method. 73
- Alumina in water....... 80
- Amido-benzol test. 42
- Ammoniated silver nitrate. 134
- **Analyses.**
  - Cow's milk. 98
  - Feces. 58
  - Gastric contents. 56
  - Human milk. 98
  - Water. 79
- Anerobic cultures.......153
- Animal inoculation......168
- Antipyrin. 142
- Ascitic fluid as culture medium. 149
- **Ash,**
  - In milk............... 84
  - In urine..............105
- Atomic weights.........175
- Babcock method........ 85
  - For human milk...... 99
- **Bacteria,**
  - Anerobic cultures.....153
  - Counting. 152
  - Culture of anerobes.....153
  - Gas production..........151
  - Hanging drop...........161
  - Motility. 161
  - Staining. 161
  - Wet preparation........161
  - In milk............... 93
    - Number of.......... 94
    - Standards of.......... 94
  - In water............. 83
  - Isolation of...........194
  - Liquefaction. 151
  - Stab culture..........151
  - Streak culture........151
- Barber's method........149
- B. Coli types............154
- **B. Coli and B. Typhosus.**
  - Adami and Chapin's method. 157
  - Drigalski-Conradi method. 158
  - Method of Hiss.......156
  - Method of Remy......153
  - Piorkowski's method..157
- **B. Typhosus.**
  - Caffein enrichment method. 159

- In feces. 159
- Loeffler's malachite green. 159
- Method of Remy......155
- Widal reaction........160

B. Tuberculosis.
- In milk............... 93
- In urine..............144
- In sputum............162
- In exudates. 36

Benzoic acid in milk.... 92
Bicarbonates. 74
Bile acids............... 61
- Pettenkofer's test...... 62

Bile.
- Gmelin's test..........138
- Hay's test............139
- In feces.............. 57
- In urine..............138
- Iodin test.............138

Bismark brown. 179

Blood.
- Alkalinity of.......... 21
- Chenzinsky's method.. 33
- Differential count..... 28
- Engel's mehotd....... 21
- Eosin and methylene blue stain. 32
- Filaria in. 33
- Guiac test. 145
- In feces. 57
- In gastric contents.....53
- In urine. 145
- Normal number of cells 28
- Percentage of leucocytes. 28
- Serum. 148
- Smears. 30, 31
- Specific gravity....... 21

Blood diluting solutions.
- Hayem's. 182
- Toisson's mixture......182

Boas' reaction.......... 45
- Resorcin test. 42
- Test breakfast. 54

Body fluids. 34
Bogg's modification. 99

Bone.
- Decalcified. 16
- Marrow. 34
- Sectioning. 14

Bouillon. 146
Bromides. 142
Buerger's method. 163
Butyric acid. 48
- In feces. 61

Calcium, in water....... 80
- Turbidity method. 77

Calcium and magnesium 113
- Carbonates and bicarbonates. 73
- Titration method. 74

Celloidin.
- Imbedding in. 11
- Preparaiont of. 11

Centrifugation of urinary sediments. 143
Centrifuge. 104

Cerebro-spinal fluid.
- Collection. 37
- Cultures from. 37
- Staining. 37

Cart f hloeucocytes...... 30
Chenzinsky's method for blood. 33
- Stain. 33

Chlorin.
- Titration method. 72
- Field method. 71
- In urine. 105
  - Gravimetric. 107
  - Neubauer-Salkowski method. 109
  - Salkowski-Volhard method. 109
  - Purdy's method. . ..105
  - Titration method. . .108
- In water. 71

Cholesterin. 62
Coagulation of water.... 82
Cochineal. 116

Coefficient
- Of fineness. 71
- Of turbidity. 71

Color
- Of water. 78
- Of urine. 101

Combined HCl. 50
Common cover glass preparation. 161
- Concretions in feces... 57

Cook's method. 133
Counting bacteria. 152
- Blood corpuscles. 28

Culture media.
- Agar-agar. 147
- Ascitic fluid. 149
- Blood serum. 148
- Bouillon. 146
- Cystic contents. 149
- Gelatin. 146
- Milk. 148
- Potato. 148
- Special variations. . ..148

Dairies, score of......94, 95
Dare's method for hemaglobin. 26
Decinormal equivalents.. . . . 186, 187, 188, 189
De Jong's method...... 47

Differential density method
- For glucose. 128

- For urea. 135

Diazo reaction. 138

Diacetic acid.
- Gerhardt's test........141
- Lindermann's test.....141
- When test for.........142

Diastasic ferment. 38

Differential count.
- Of erythrocytes 28
- Of leucocytes. 29, 30

Digesting power
- Of saliva. 38
- Of gastric juice. . . . . .

Diphtheria. 166
- At bedside. 166
- In laboratory. 167
- Neisser's stain. 167
- Of vulva. 40

Doremus-Hinds ureometer. 134, 135

Drigalski-Conradi method. 158
- Hagemann's modification. 158

Drug poisoning.
- Acetanilid. 142
- Phenacetin. 142
- Antipyrin. 142
- Bromides. 142
- Iodin. 143

Ehrlich's triple stain....181

Engel's method. 21

Eosin. 178

Eosin and methylene blue. 32

Erythrocytes. 28

Esbach's method for albumin. 119

Ewald-Sievers method... 55

Ewalds' test breakfast.. 54

Exudates. 36
- Chylous. 36
- Hemorrhagic. 36
- Inoscopy. 36
- Method of Jousset.... 36
- Purulent. 36

Fat
- In chylous exudaets... 36
- In milk. 85
  - Babcock method.... 85
- Ether extraction methods. 86
- Feser's method. 85
- Leffman-Beam method 99
- Stains for. 14

Fatty acids.
- Cahn-Mehring-McNaught method... 51
- In feces. 60
- In gastric contents.... 51

Feces,
- Acetic acid in.......... 60
- Bile acids in.......... 61
- Bile in. 57
- Blood in. 57
- Butyric acid in........ 61
- Cholesterin in. 62
- Concretions in. 57
- Examination of. 56
- Fatty acids in......... 60
- Foreign bodies in...... 57
- Formic acid in........ 60
- Indol in. 59
- Microscopic examination. 63
- Parasites in. 57
- Phenol in. 59
- Skatol in. 60
- Valerianic acid in..... 61

Fehling or Haines test..124

Fehling's test for glucose 125

Fermentation test for glucose. 127

Ferric ammonium sulfate. 185

Feser's method. 99

Filaria in blood......... 33

Filtration
- Of culture media..147, 148
- Of urinary sediments..143
- Of water. 83

Fineness, coefficient of... 71

Folin's mehtod for uric acid. 132

Foreign bodies in feces.. 57

Formaldehyd in milk.... 91
- Hydrochloric acid test 91
- Sulfuric acid test...... 92

Formic acid in feces.... 60

Fowler's method........135

Free HCl. 49

Gas Formula. 152

Gas production,
- In liquid media.......152
- In solid media........151

Gastric contents,
- Acetic acid in......... 49
- Amido benzol test.... 42
- Blood in. 53
- Butyric acid in....... 48
- Combined HCl. 50
- Congo-red test. 42
- Fatty acids in........ 51
- Free acids in.......... 42
- Free HCl. 42
- Lactic acid in......44, 51
- Mohr's test. 44
- Organic acids in...... 50
- Pepsin and pepsinogen. 47
- Phloroglucin-Vanillin test. 43
- Qualitative tests. 42
- Quantitative estimations. 49

Rennin. 48
Renninogen. 48
Resorcin test. 42
Total acidity. 49
Tropoeolin test. 43
Gelatin. 146
Gerhardt's test for diacetic acid. 141
Germicides. 173
Giemsa's stain. 167, 168
Globulin. 123
Glucose in urine.
Differential density method. 128
Fehling's method. 125
Quantitative. 126
Fermentation method. 127
Haines' method. 125
Polarization. 128
Purdy's method. 126
Quantitative estimation. 126
Gmelin's test for bile. . . .138
Gonorrhoea. 166
In female. 40
Gower's method for hemaglobin. 24
Gram negative bacteria. .172
Gram positive bacetria. .172
Gram's method. 163
Gram's solution. 163
Gravimetric methods:
For albumin. 120
For chlorides. 107
For phosfates in urine.115
For total sulfates. . . .111
For water analysis. . . .
Guiac test for blood. . . .145
Gumprecht's table. 23
Gunning's test for acetone. 139
Gunzburg's method. 55
Hagemann's modification 158
Haines' test for glucose. .125
Hammerschlag's table. . . 22
Hanging drop. 161
Hardness of water. 72
Sodium oleate method. 72
Hayem's solution. 182
Hay's test. 139
Hazen's scale. 65
HCl, combined. 50
Heintz's method. 133
Heller's test. 117
Hemacytometer. 29
Hemaglobin.
Dare's method. 26
Gower's method. 24
Sahli's method. 25
Tallquist method. 24
Von Fleischl-Meischer method. 27
Hematoxylin. 178
Hematoxylin and eosin.
For blood. 31
For tissue. 12
Human milk. 98
Amount. 98
Analyses of. 98
Babcock method. 99
Boggs' modification. . . . 99
Leffman-Beam method. 99
Proteids. 99
Woodward's method. . 99
Imbedding
In celloidin. 11
In paraffin. 20
.g 7d4 . . . .
Immersion refractometer 11
Indicators. 184
Ferric ammonium sulfate. 185
Litmus. 184
Methyl orange. 185
Phenolphthalein. 185
Potassium chromate. . .185
Potassium dichromate.186
Rosolic acid. 185
Starch solution. 186
Indol in feces. 59
Inoculaiton of animals. .168
Iodin. 143
Test for bile. 138
Iron in water. 79, 80
Isolation of bacteria.
Barbers' method. 149
Plate method. 149
Jackson's turbidimeter. . 69
Jenner's. 180
Jolle's test for albumin 119
Jousset, method of. 36
Kelling's test. 45
Knapp's method. 47
Kjeldahl method. 136
Lactic acid. 44
Boas' reaction. 45
De Long's method. 47
In gastric contents. . . . 51
Kelling's test. 45
Knapp's method. 47
Strauss' test. 45
Uffelmann's test. 44
Lactodensimeter. 98
Lange's test for acetone.140
Leffman-Beam method. . 99
Leube's method. 55
Leucocytes. 28, 29, 30
Lieben's test for acetone.139
Lindermann's test. 141
Liquefaction. 151
Litmus. 184
Loeffler's method. 164
Ludwig's magnesium mixture. 182

- Lugol's solution. 182
- Magnesia in water. 80
- Malachite green. 159
- Malaria, thionin stain for 32
- Menstrual fluid. 41
- **Method:**
  - Adami and Chapin. . . .157
  - Barber's. 149
  - Buerger's. 163
  - Caffein enrichment. . . 159
  - Cahn-Mehring-McNaught. 51
  - Common cover glass. . .161
  - Cook's. 133
  - De Jong's. 47
  - Differential density. . . .128
  - Drigalski-Conradi. . . .158
  - Engel's. 21
  - Ewald-Sievers. 55
  - Fermentation for glucose. 127
  - Folin's. 132
  - For gonorrhea. 166
  - Fowler's. 135
  - Gram's. 162
  - Gunzburg's. 55
  - Hagemann's modificaiton. 158
  - Hehner-Seeman. 50
  - Heintz's. 133
  - Hiss'. 156
  - Jackson's turbidimeter. 70
  - Jousset. 36
  - Kjeldahl. 136
  - Knapp's. 47
  - Leube's. 55
  - Loeffler's. 164
  - Malachite green. 159
  - Neubauer-Salkowski. . 109
  - Piorkowski's. 157
  - Purdy's. 105, 110, 113, 121, 126
  - Remy. 155
  - Ruhemann's. 129
  - Salkowski-Volhard. . .109
  - Smith's. 164
  - Sodium oleate. 72
  - Topfer's. 49
  - Van Ermengem. 165
  - Ziehl-Neelson. 162
- **Methods of Hiss:**
  - For tubes. 156
  - For plating. 156
- Methyl green. 181
- Methyl orange. 185
- Methylene blue. 179
- Microscopio examination
  - Of feces. 63
  - Of sediments. 35
- **Microtome:**
  - Cutting with. 12
  - Freeinzg. 14
- **Milk:**
  - Added water. 90
  - As culture medium. . .148
  - Bacteria in. 93
  - B. Tuberculosis in. . . . 93
  - Benzoic acid in. 92
- Milk depots, score of. . . . 96
- Milk, formaldehyd in. . . . 91
- Milk, human. 98
- Milk, Salicylic acid in. . . 93
- Milk serum. 90
- Milk, skimmed. 90
- Milk stores, score of. . . . 97
- Milk, whole. 91
- Mohr's test. 44
- **Motility:**
  - Of bacteria. 161
  - Of spermatozoa. 39
- Motor power of stomach. 55
- Muller's fluid. 182
- Nasal secretion. 38
  - Microscopic examination. 38
  - Reaction. 38
  - Taste. 38
- Neubauer-Salkowski method. 109
- Nitric acid contact test. .117
- **Nitrogen in urine**
  - Kjeldahl method. 136
- Normal and decinormal solutions. . . .183, 184
- Opsonic index. 169
  - An example. 172
  - Counting. 171
  - Emulsion. 169
  - Index. 171
  - Serum. 170
  - Smear. 171
  - Staining. 171
  - Washed blood corpuscles. 169
- Organic acids. 50
  - Hehner-Seeman method 50
- Oxalic acid in urine. 137
- Pal solution. 17
- Paraffin imbedding. 20
- Parasites in feces. 57
- Parke's table. 103
- Peiper, Schmaltz and. . . . 22
- Pepsin. 53
- Petri dish. 149
- Phenacetin. 142
- Phenol in feces. 59
- Phenolphthalein. 185
- Phloroglucin-vanillin test 43
- **Phosfates in urine:**
  - Gravimetric methods 115
  - Of calcium and magnesium. 113
  - Purdy's method. 113
  - Sodium and potassium 114

  - Titration methods 116, 117
- Picro-carmin. 181
- Piorkowski's method....157
- Plate cultures. 149
- Polarization. 128
- Post mortem bacteriology. 169
- Potassium ferrocyanid and acetic acid test....118
- Potassium chromate. . ..185
- Potassium dichromate. . .186
- Potassium in water..... 81
- Potassium and sodium... 82
- Potato as culture medium 148
- Precipitation of water... 82
- Preparation of sections.. 12
- Preparation of tissue.... 11
- Proteids in human milk 99
- **Purdy's methods:**
  - For albumin. 121
  - For chlorides. 105
  - For glucose. 126
  - For phosfates. 113
  - For sulfates. 110
- Purdy tube. 105
- Purin bodies. 133
  - Cook's method. 133
- Pus. 166
  - In the eye........... 39
- **Reaction:**
  - Blood. 21
  - Feces. 58
  - Gastric contents. 42
  - Human milk. 98
  - Nasal secretion. 38
  - Saliva. 38
  - Urine. 103
  - Vaginal secretion. 40
- Reagents, sensitive to HCl. 56
- Red bone marrow....... 34
- Refractometer, immersion 91
- Reigel's test dinner..... 54
- Remy's method..........156
- Rennin. 48
- Reninogen. 48
- Resorcin test. 42
- Rice's solutions. 134
- Romanowski's stain. . ..179
- Rosolic acid. 185
- Ruhemann's method. . ..129
  - Modification No. 1.....129
  - Modification No. 2.....130
- Safranin. 181
- Sahli's method. 25
- Salicylic acid in milk.... 93
- Saliva, amount. 38
  - Collection. 37
  - Digesting power. 38
  - Ferment. 38
  - Reaction. 38
  - Specific gravity. 38
- Salkowski-Volhard method. 109
- Salzer test meal. 54
- Sample of urine. 101
- Schmaltz and Peiper.... 22
- Score of milk depots.... 96
- Score of milk stores.... 97
- Scoring dairies. 94, 95
- **Sections:**
  - Cutting. 12
  - Frozen. 14
  - Mounting. 12
  - Of bone. 14
  - Staining. 12
  - Unstained. 16
- Seminal fluid, collection.. 39
  - Motility. 39
  - Staining. 39
- Sewage bacteria. 154
  - Coli types. 154
  - In water. 83
- Silica in water. 79
  - Standard. 70
- Simple still. 58
- Skatol in feces. 60
- **Smears:**
  - Blood. 10, 31
  - Material of abortion... 41
  - Menstrual fluid. 41
  - Nasal secretion. 38
  - Sediments. 35
  - Seminal fluid. 39
  - Vaginal secretion. 40
- Smith's method. 164
- **Sodium and potassium:**
  - In urine. 114
  - In water. 81
  - Separation of 82
- Sodium hypochlorite method. 135
- Sodium oleate method... 72
- Softening of water. . .. 83
- **Solids:**
  - In blood. 23
  - In milk. 84
  - In urine. 103, 104
  - In water. 71
- **Solutions,**
  - Ammoniated silver nitrate. 134
  - Barium chlorid mixture. 110
  - Destaining solution. . 162
  - Diazo. 138
  - Esbach's. 121
  - Fehling's. 125
  - Folin's. 132
  - Gram's. 163
  - Haines'. 125
  - Hayem's. 182

  - Jolle's. 119
  - Jousset's. 36
  - Ludwig's mag. mix. . . . 182
  - Lugol's. 182
  - Muller's fluid. 182
  - Official dil. HCl. 183
  - Purdy's. 126, 127
  - Rice's. 134
  - Ruhemann's. 129
  - Sodium acetate. 116
  - Spiegler's. 118
  - Tanret's. 118
  - Toisson's mix. 182
  - Uranium nitrate. 116
- **Specific gravity,**
  - Beads. 192
  - Hammerschlag's table. . 22
  - Of blood. 21
  - Of exudates. 36
  - Of human milk. 98
  - Of milk. . . . .
  - Of saliva. 38
  - Ruess' table. 34
  - Schmaltz and Peiper. . 22
  - Transudates. 34
  - Urine. 102
- Spermatozoa. 39
- Spiegler's test. 118
- Splenic pulp. 34
- Stab culture. 151
- Staining bacteria. 161
  - Common cover glass. . . 161
  - Gram's method. 162
  - Ziehl-Neelson method. 162
- **Staining blood**
  - Chenzinsky's method. . 33
  - Ehrlich's method. . . . 32
  - Eosin and methylene blue. 32
  - Hematoxylin and eosin 32
  - Thionin. 32
  - Wright's method. . . . 32
- **Staining capsules**
  - Buerger's method. 163
  - Smith's method. 164
- **Staining fat:**
  - Osmic acid. 14
  - Sudan III. 14
- **Staining flagella :**
  - Loeffler's method. . . . 164
  - Van Ermengem's. method. 165
- **Staining:**
  - Nasal secretions. 38
  - Pus from eye. 39
- **Staining sections.**
  - Combined process. . . . 20
  - Golgi method. 17
  - Hematoxylin and eosin 12
  - Intra vitam. 18
  - Nissl's method. 19
  - Silver nitrate method. . 13
  - Van Gieson's method. . 13
  - Weigert-Pal method. . . 16
- Staining seminal fluid. . . 39
- Staining spores 166
- **Stains:**
  - Bismark brown. . . . 179
  - Chenzinsky's. 33
  - Congo red. 178
  - Ehrlich's triple. 181
  - Eosin. 178
  - Golgi. 17
  - Hematoxylin. 178
- Jenner's 180
  - Intra vitam. 18
  - Loeffler's methylene blue. 179
  - Methyl green. 181
  - Neisser's. 167
  - Nissl's. 19
  - Picro-carmin. 181
  - Romanowsky's. 179
  - Safranin. 181
  - Thionin. 32
  - Van Gieson's. 181
  - Weigert. 16
    - Elastic tissue. 19
  - Wright's blood. 180
  - Ziehl's solution. 179
- Standardizing solutions. . 184
- Starch solution. 186
- Still. 58
- Strauss' test. 45
- **Sugar:**
  - In chylous exudates. . 36
  - In milk. 89
  - In urine. 125
- Sulfates . . . .
  - Turbidity method. . . . 75
- **Sulfates in urine,**
  - Ethereal. 121
  - Mineral. 112
  - Purdy's method. 110
  - Total sulfates. 111
- Sulfur in urine. 112
- Sulfuric acid in water. . . 80
- **Table of**
  - Albumin. 121
  - Atomic weights. 175
  - Burgess. 173
  - Calcium. 77
  - Chlorides. 106
  - Germicides. 173, 174
  - Gumprecht. 23
  - Hammerschlag. 22
  - Parke. 103
  - Phosfates. 114
  - Ruess. 34
  - Ruhemann. 130
  - Schmaltz and Peiper. . 22
  - Sulfates in urine. 110
    - In water. 75
- Tablets. 183

Tallquist method. 24
Tanret's test. 118
Tartaric acid N-75 21
Test breakfast, Boas'. . . . 54
  Ewald's. 54
Test dinner, Reigel's. . . . 54
Test meal, Salzer. 54
Thionin stain. 32
Thoma-Zeiss hemacytometer. 29
Tissue. 11, 16
Titration. 108
Titration Methods:
  Albumin. 120
  Alkalinity. 73
  Acidity. 49, 50
  Carbonates. 74
  Chlorides. 108
  chlorin. 72, 108
  Glucose. 126
  Milk sugar. 89
  Phosfates. 116
  Uric acid. 129
Toisson's mixture. 182
Total acidity. 49
Transudates. 34
Treponema pallidum. . . .167
Trommer's test, acetone.140
Tropaeolin test. 43
Trypanosoma. 33
Tuberculosis:
  In exudates. 36
  In milk. 39
  In sputum. 162
  In urine. 144
Turbidimeter:
  Hazen's scale. 65
  Jackson's. 69
  U. S. Geol. survey rod. 67
Turbidity.
  Coefficient of. 71
  In filield work. 70
  Silica standard. 70
Uffelmann's test. 44
United States Geol. Survey rod. 67
Uranium nitrate. 116
Urates. 145
Urea.
  Fowler's method. 135
  Hypobromite method. .135
Ureometer. 134
  Doremus-Hinds. 134
Uric acid.
  Folin's method. 132
  Heintz's method. 133
  Ruhemann's method. . 129
  With Esbach's Sol. 133
Urinalysis. 101
Urinometer. 102
Urinary sediments
  By centrifugation. . . .143
  Examination. 143
  By filtration. 143
Urine
  Acetone in. 139
  Albumin in. 117
  Amorphous urates. . . .145
  As culture medium. . . .149
  Ash in. 105
  Bile in. 138
  Blood in. 145
  Chemical examination.103
  Chlorides in. 105
  Color (Plate I). 101
  Diacetic acid in. 141
  Diazo reaction. 138
  Drug poisoning in. 142
  Ethereal sulfates. . . .112
  Glucose in. 125
  Mineral sulfates. 112
  Nitrogen in. 136
  Oxalic acid in. 137
  Phosfates in. 113
  Physical examination. .101
  Purin bodies. 133
  Reaction. 103
  Solids in. 103
  Specific gravity of. 102
  Sulfates in. 110
  Total sulfur. 112
  Tuberculosis in. 144
  Sediments. 143
  Urea in. 134
  Uric acid in. 129
  Vogel's scale. . . .Plate I
Vaginal secretions:
  Examination 40
  Reaction. 40
Valerianic acid in feces. 61
Van Ermengem's method 165
Van Gieson's stain. 181
Vogel's scale. . . . .Plate I
Von Fleischl-Meischer. . . 27
Water added to milk. . . . 90
  Alkalinity. 73
  Alumina in. 80
  Analysis of. 79
  Bacteria in. 83
  Bicarbonates in. 73
  Calcium in. 77. 80
  Carbonates in. 73
  Chlorin in. 71
  Coagulation of. 82
  Color. 78
  Examination of. 65
  Filtration. 83
  Hardness. 72
  Iron in. 79, 80
  Magnesia in. 80
  Precipitation. 82
  Potassium in. 81

  Sodium in. 81
  Softening. 83
  Solids in. 71
  Sewage in. 83
  Silica in. 79
  Sulfates in. 75
  Sulfuric acid in....... 80
  Turbidity. 65

Wet preparation

  Of bacteria. 161
  Of vaginal secretion... 40

Whole milk. 91

Widal reaction. 160

Woodward's method. . .. 99

Wright's blood stain. . ..180

Zeiss immersion refractometer. 91

Ziehl-Neelson method. . .162

Ziehl's solution. 179